Genome evolution of fungal pathoens from Magnaporthe oryzae/grisea clade

Marc-Henri M.-H. Lebrun, Ludovic Mallet, Cyprien Guerin, Hélène Chiapello, Enrique Ortega-Abboud, Annie Gendrault-Jacquemard Gendrault, Jonathan J. Kreplak, Thomas T. Kroj, Arnaud Couloux, Corinne Cruaud, et al.

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IS-MPMI 2012 XV International Congress

PROGRAM AND ABSTRACTS

July 29 (Sun.) –August 2 (Thu.), 2012 · Kyoto, Japan

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Welcome

Letter from Organizing Committee Chair

Welcome to Kyoto and the XV International Congress on Molecular Plant-Microbe Interactions

It is my great pleasure to receive more than 1000 scientists and students from all over the world in Kyoto for the IS-MPMI 2012. We have been able to make an outstanding program consisting of plenary lectures, concurrent sessions, poster sessions, and special workshops. In addition, we will have an opening lecture by a renowned Japanese immunologist, Prof. Shizuo Akira. I am confident that all the participants will have great opportunities for exchanges of research, ideas and finding old and new friends during the congress.

I have three points to make concerning the preparation of this congress. First, I have to mention the disastrous earthquake which occurred in the North-East part of Japan on March 11, 2011. Although Kyoto is quite far from the areas where the earthquake hit, there were many concerns among the local and international MPMI communities on whether we should have the congress in the summer of 2011 as originally planned. After extensive discussions we eventually decided to postpone the congress one year. It was a difficult decision but now I feel that it was a right decision.

Second, this is the first IS-MPMI congress taking place outside America and Europe. To make IS-MPMI a true international organization and for the advance of MPMI research in the future this congress would become a milestone for the future of international MPMI research. I really hope that Asian scientists will experience exciting research in the field, get stimulation and find friends during the congress.

Finally, I hope that all the participants will enjoy Kyoto which is one of the most attractive cities in Japan and you can find every Japanese culture and tradition here. This is exactly the reason for us to decide to have IS-MPMI 2012 in this city.
Thank you

Ko Shimamoto
Chair, The Organizing Committee of IS-MPMI 2012

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The Molecular Biology Society of Japan
The Japanese Society of Plant Physiologists
The Phytopathological Society of Japan
Pesticide Science Society of Japan
Japanese Society of Breeding
Japanese Society of Plant Microbe Interactions

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GENERAL INFORMATION

Venue
Kyoto International Conference Center (ICC Kyoto)
Takaragaike, Sakyo-ku, Kyoto 606-0001
Phone: + 81-75-705-2001
(Congress Secretariat *July 29–August 2 only)
URL: http://www.icckyoto.or.jp/en/index.html

Registration
The registration desk is located at the entrance of the ICC Kyoto. Advance and on-site registrants may pick up their congress material upon registration.

Registration Hours

Sunday, July 29	10:00 - 20:00
Monday, July 30	7:30 - 17:00
Tuesday, July 31	8:00 - 17:00
Wednesday, August 1	8:00 - 15:30
Thursday, August 2	8:00 - 12:00

On-site Registration Fee
For those who wish to register after July 4, 2012, please register at the on-site registration desk. Cash or Credit cards, as shown below, are available for payment of on-site registrations: Visa, MasterCard, American Express, Diners, JCB, and Nicos.

IS-MPMI member	JPY	78,000
Non-member	JPY	88,000
Student	JPY	34,000
Accompanying person	JPY	10,000

Name Badge
All participants, accompanying persons, and exhibitors are asked to wear the provided name badge in the congress areas. Replacement of lost badges will incur a full charge. Accompanying persons CANNOT attend any scientific sessions.

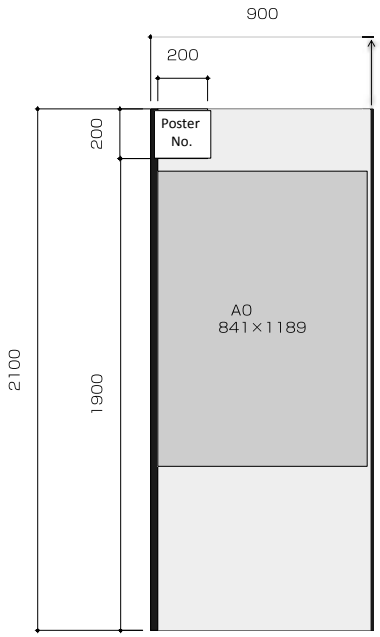
Instructions for Oral Presentations

- Presenters must gather in their session room **15 minutes prior to the session start time** to conduct a brief meeting to coordinate the session.
- Please bring your own computer, and ensure that your computer is equipped with the proper monitor connector (mini D-sub15 pins), as shown below. If your computer does not have this connection, please bring an appropriate converter with you.

- During a meeting, moderators work with presenters to make sure the presentations are ready for projection onto a screen.
- The organizing committee DOES NOT prepare computers for oral presentations.
- The time for plenary sessions is 30 minutes each, including discussion; special workshops and concurrent sessions should be within 20 minutes each, including discussion.

Instructions for Poster Preparation

- Posters will be displayed in the Event Hall of ICC Kyoto.
- Posters are numbered as indicated on page 11 and the sigh of the Event Hall, and a corresponding numbered poster board is available for attaching your poster.
- Posters still displayed after the removal time has passed will be disposed of by the Congress Secretariat.
- Authors are expected to be at their poster during the assigned sessions.
- Please make your poster fit the space within the display panel. The panel size is shown in the figure below.



(Unit: mm)

Poster Hours

Sunday, July 29	14:00 - 17:30	*Poster set-up*
Monday, July 30	8:30 - 20:30	*Poster viewing*
	12:20 - 13:30	*Poster set-up*
	18:00 - 20:00	*Odd-numbered posters: authors present*
Tuesday, July 31	8:30 - 20:30	*Poster viewing*
	18:00 - 20:00	*Even-numbered posters: authors present*
Wednesday, August 1	8:30 - 17:30	*Poster viewing*
Thursday, August 2	8:30 - 12:20	*Poster viewing*
	12:20 - 13:30	*Poster take-down*

GENERAL INFORMATION *(continued)*

Exhibit Hours

Sunday, July 29	14:00 - 17:30	*Exhibitor set-up*
Monday, July 30	8:30 - 20:30	*Exhibits open*
Tuesday, July 31	8:30 - 20:30	*Exhibits open*
Wednesday, August 1	8:30 - 17:30	*Exhibits open*
Thursday, August 2	8:30 - 12:20	*Exhibits open*
	13:30 - 15:00	*Exhibitor move-out*

Information Desk and Tour Desk

Information and Tour Desk are located on the 1st floor of ICC Kyoto. They will be open during the following times:

Sunday, July 29	11:00 - 20:00
Monday, July 30	10:00 - 17:00
Tuesday, July 31	10:00 - 17:00
Wednesday, August 1	10:00 - 16:00
Thursday, August 2	10:00 - 17:00

Lunch

Lunch boxes are to be provided by the organization committee in exchange for lunch vouchers only; they are not to be sold. If you are a vegetarian, choose a vegetarian lunch when registering. Your choice of lunch cannot be changed on site.

Drink Ticket at Poster Sessions

In exchange for drink tickets, free drinks are available at the Event Hall while Poster Sessions are held from 18:00 to 20:00 on July 30 and 31. A glass of beer, wine or juice will be served for one ticket. Drink tickets will be provided with your name badge. If you use up all the tickets, you can pay in cash for another drink. Note the drink tickets can not get cashed.

Excursion

[Venue]	World Heritage "Kiyomizu-dera" & Gion Hanamikoji dori (requires advance registration)
[Date & Time]	**Wednesday, August 1, 2012, 16:00**
[Pick-up Location]	In front of the main entrance of ICC Kyoto
[Schedule]	16:00: ICC Kyoto 16:40: Kiyomizu-dera (1-hour guided tour & free time) 18:00: Gion Hanamikoji dori (City view from window) Stop by hotels in Kyoto 20:00: Grand Prince Hotel Kyoto

The organizing committee will prepare some seats of the Excursion for on-site registration. If the tour reaches its maximum capacity, it will be unavailable.

Congress Dinner

An optional Congress Dinner will take place at the Grand Prince Hotel Kyoto on August 2, 2012, from 7 p.m. to 9 p.m. The Grand Prince Hotel Kyoto is a 5-minute walk from ICC Kyoto. Advanced reservations must be obtained via our website by July 3. We are unable to accept on-site applications.

Grand Prince Hotel Kyoto
Takaragaike, Sakyo-ku, Kyoto 606-8505
Phone: 075-712-1111 Fax: 075-712-7677
URL: http://www.princehotels.co.jp/kyoto/

Internet Connection

Free WiFi access is provided in most corridors but not in the inside rooms of ICC Kyoto. No special security measures have been put in place.
SSID: ICCK_Public_WiFi
See page 9 for more information on the free WiFi spots.

Social Media

The organizing committee encourages the use of social media before, during, and after the meeting. Please follow these guidelines:

- Follow us on Twitter @MPMIKyoto and @ ismpmi. Use the hash tags #MPMI2012 for meeting–related tweets.
- Follow us on Facebook:
  XV congress: http://www.facebook.com/mpmi.kyoto
  IS-MPMI: https://www.facebook.com/ISMPMI
- Blog about the meeting and what you are hearing and seeing (but without sharing details of any data presented).

Business Center

The business center is located on the 1st floor of ICC Kyoto. Copy machines, computers, and access to the Internet will be available. Express deliveries are also available.

Cloakrooms

Cloak 1 near the Information and Tour Desk is available up to 200 participants all the time during the congress (See page 8). Cloak 2 near the main entrance is opened additionally when Cloak 1 is full.

GENERAL INFORMATION

Prohibitions
Photography, video recording, and sound recording are prohibited within the meeting venue.

Mobile Phones
Attendees are asked to be respectful of their colleagues by turning off all cell phones and smartphones before entering meeting rooms.

Photo Release
Photographs will be taken at the XV Congress. By registering for this congress, you agree to allow IS-MPMI or the organizing committee to use your photo in any of their publications or on Facebook: http://www.facebook.com/mpmi.kyoto

Climate & Weather
Light clothing and short sleeves are suitable. Kyoto is located in a basin surrounded by mountains and experiences a warm summer.

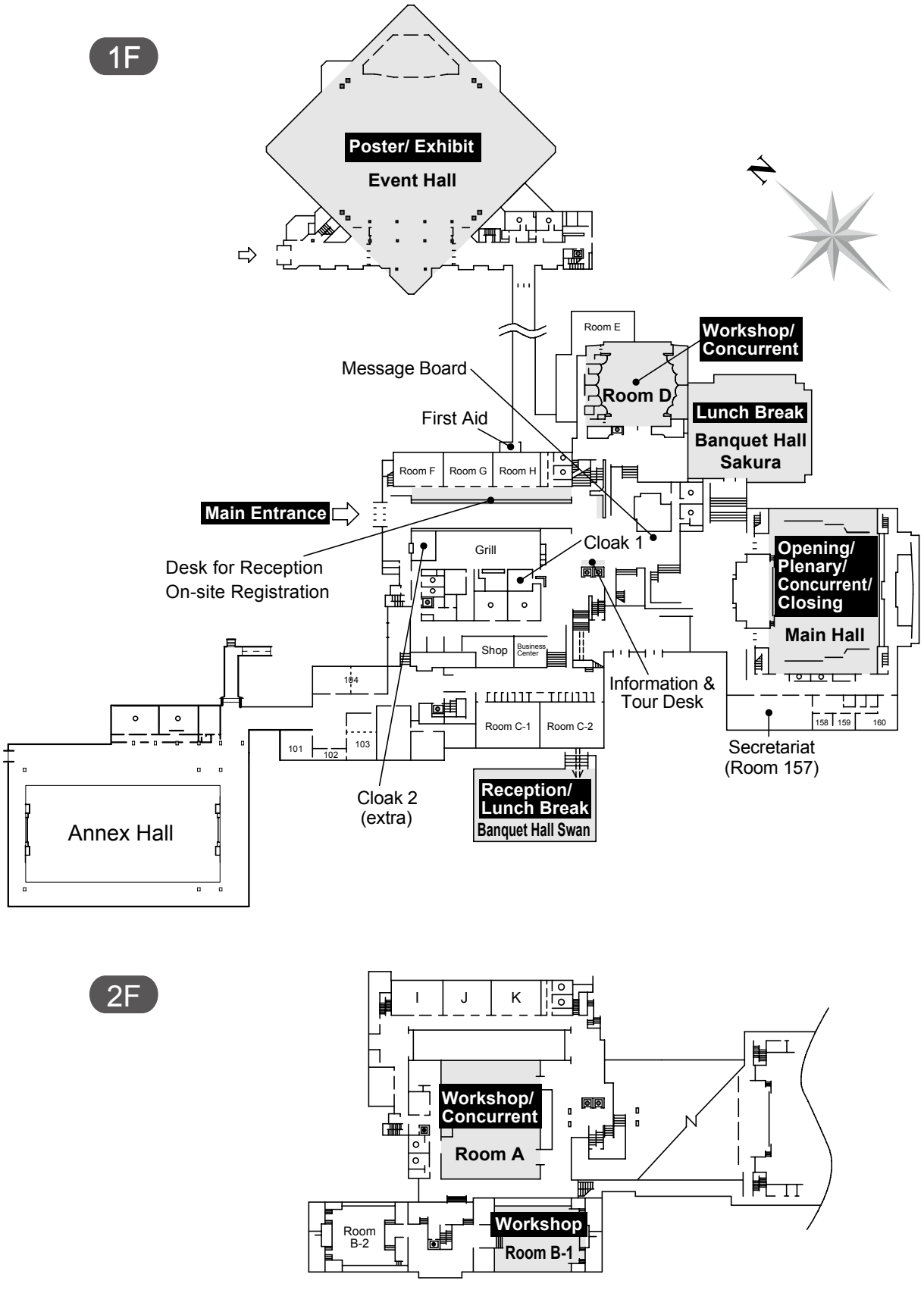
Emergencies
Medical emergencies should be communicated to a Congress Secretariat member at the registration desk or to an employee of ICC Kyoto.

Other Numbers
Dial 110 for the police and 119 for the fire department or an ambulance.
No money is required to make these calls.
International Medical Information Center Kansai: 06-4395-0555
Metropolitan Police Dept. Counseling Service for Foreigners: 03-3503-8484
For hospitals with foreign language-speaking doctors and other useful information, see the Kyoto City International Foundation website (http://www.kcif.or.jp/en/).

MAPS

Kyoto International Conference Center (ICC Kyoto)

Floor Plan

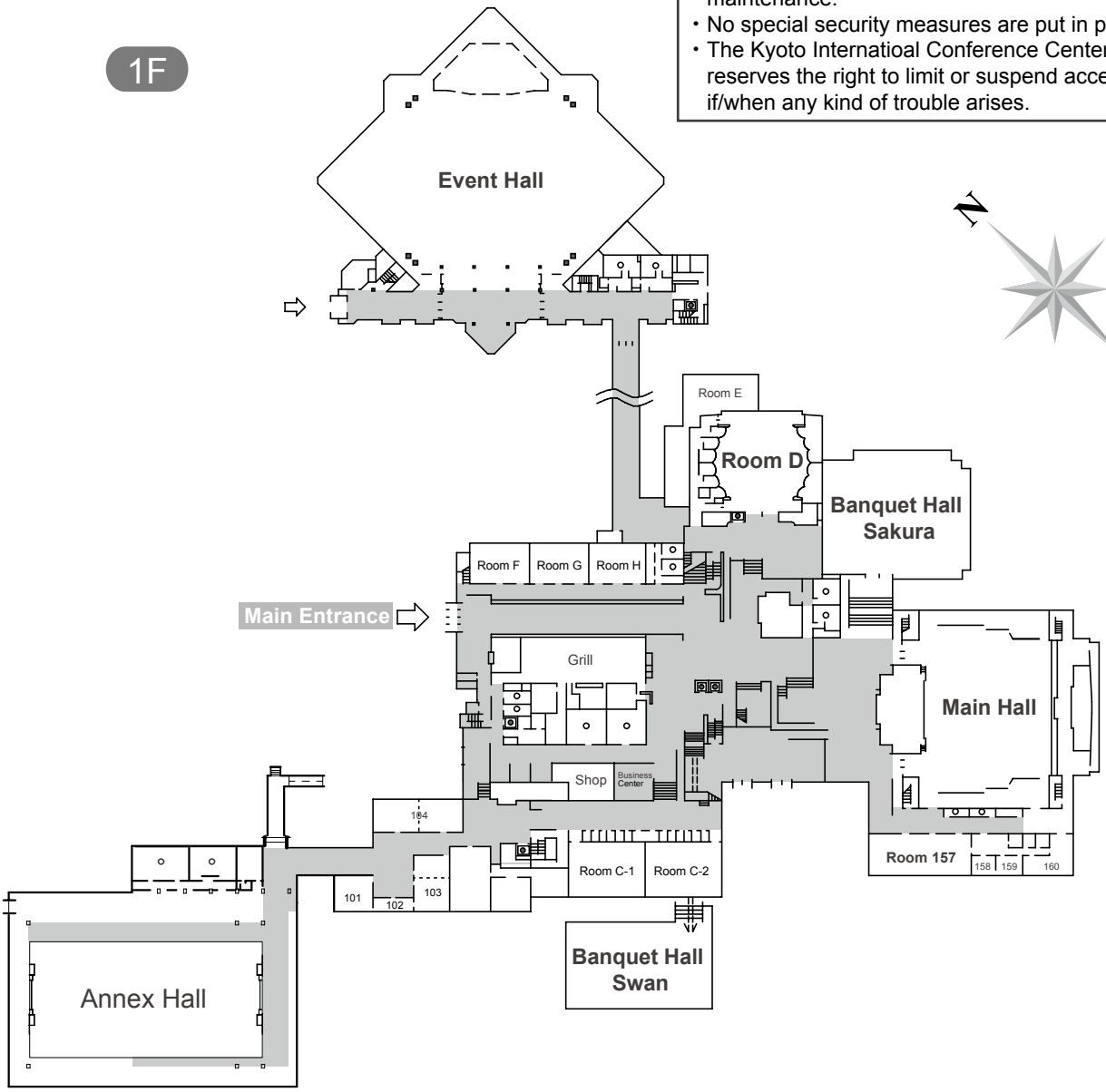


Free WiFi Area Map

SSID: ICCK_Public_WiFi

—ATTENTION—
- Users shall be responsible for any problems that may arise due to connecting and/or reconfiguring PCs.
- Services may be interrupted for server maintenance.
- No special security measures are put in place.
- The Kyoto Internatioal Conference Center reserves the right to limit or suspend access if/when any kind of trouble arises.

1F



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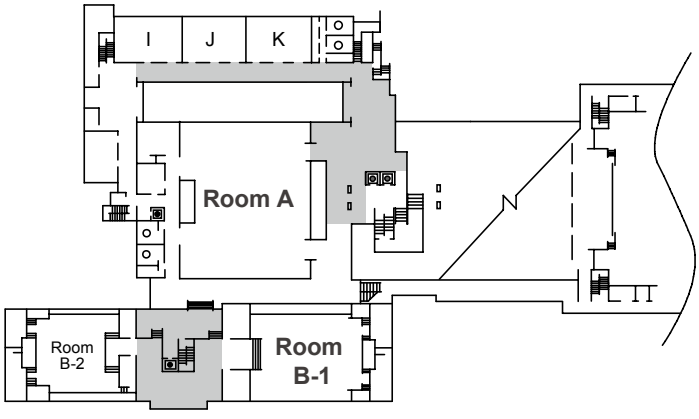
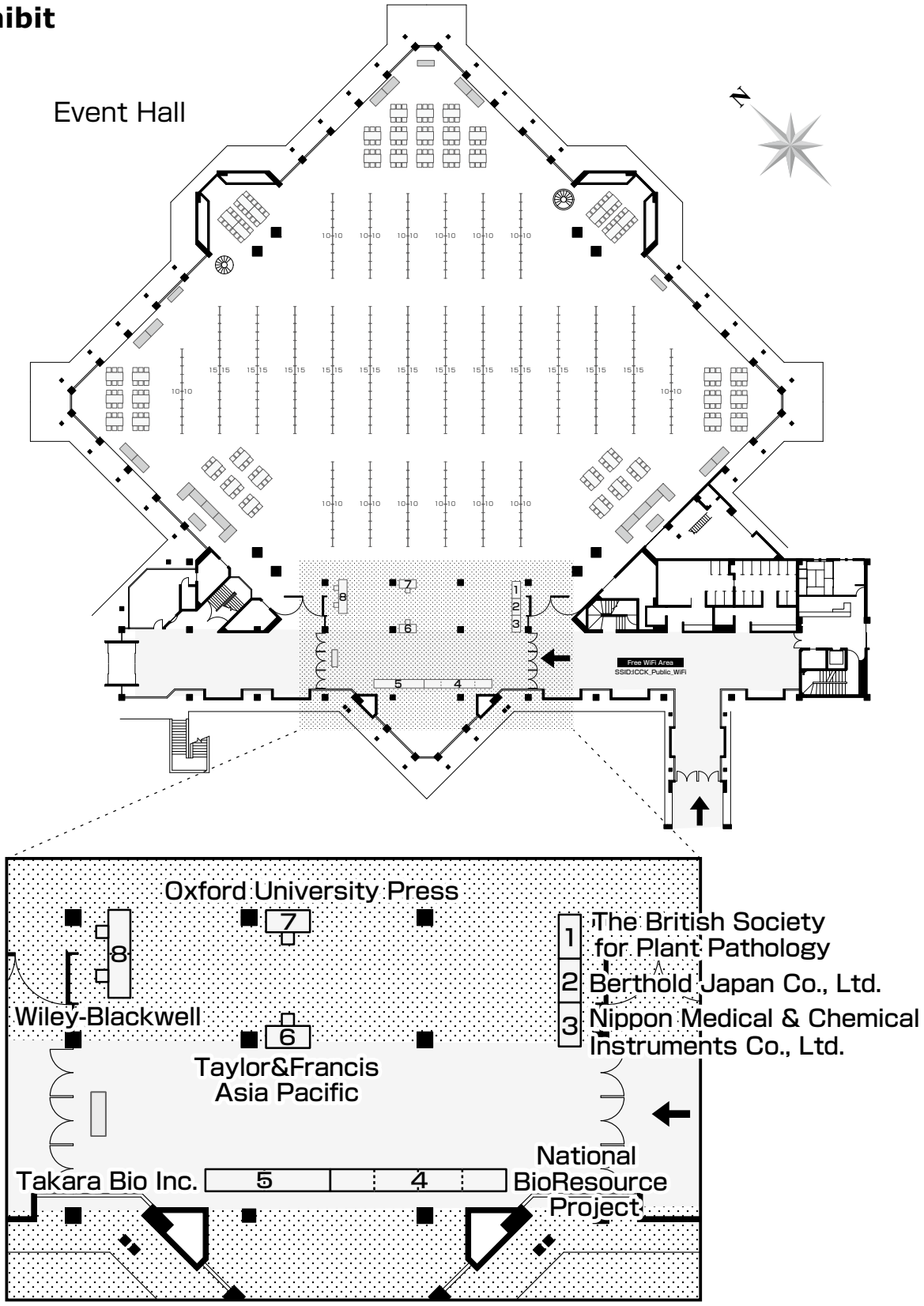


Free WiFi Area

Exhibit



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8) John Wiley & Sons, Inc.

Poster Session

2012 XV International Congress on Molecular Plant-Microbe Interactions

PROGRAM-AT-A-GLANCE

Time	Sun. July 29	Time	Mon. July 30
		8:30 - 10:00	**Plenary 1** Plant signaling I *Main Hall* Cyril Zipfel Frank Takken Ko Shimamoto
		10:00 - 10:20	**Coffee Break**
		10:20 - 12:20	**Plenary 2** Pathogen *Main Hall* Jeff Dangl Paul Schulze-Lefert Sophien Kamoun Yong-Hwan Lee
12:00 - 14:00	**Special Workshop 1** Imaging plant-microbe interactions *Room A* **Special Workshop 2** Induced susceptibility in plants *Room B-1* **Special Workshop 3** Powdery mildew *Room D*	12:20 - 13:30	**Lunch Break**
14:00 - 14:30	**Break**	13:30 - 15:30	**Concurrent 01** Recognition and signaling I *Main Hall* **Concurrent 02** Symbiosis I *Room A* **Concurrent 03** Pathogenic fungi *Room D*
14:30 - 16:30	**Special Workshop 4** Rice immunity and pathogens *Room A* **Special Workshop 5** Functional genomics of plant pathogenic bacteria *Room B-1* **Special Workshop 6** Proteomics *Room D*	15:30 - 15:50	**Coffee Break**
16:30 - 17:00	**Break**	15:50 - 17:50	**Concurrent 04** Plant-oomycete/fungal interactions *Main Hall* **Concurrent 05** Biocontrol interactions *Room A* **Concurrent 06** Plant-nematode/insect interactions *Room D*
17:00 - 19:00	**Opening Remark** **Opening Lecture** Shizuo Akira **Award Lecture** *Main Hall*	17:50 - 18:00	
19:00 - 19:15		18:00 - 20:30	**Poster Session I** (Odd number) *Event Hall*
19:15 - 21:00	**Welcome Reception** *Banquet Hall Swan*		

Attendees are welcome to view posters throughout the day, Monday and Tuesday, 8:30 - 20:30, Wednesday, 8:30 - 17:30 and Thursday, 8:30 - 12:20

Tue. July 31	Wed. August 1	Thu. August 2
Plenary 3 Plant immunity I *Main Hall* Jane Parker Jonathan Jones Pietro Spanu	**Plenary 5** Plant signaling II *Main Hall* Peter N. Dodds Jen Sheen Brian Staskawicz	**Plenary 7** Plant immunity II *Main Hall* John Rathjen Regine Kahmann Sheng Yang He
Coffee Break		
Plenary 4 Plant-microbe interactions I *Main Hall* Maria Harrison Ton Bisseling Xin Li Naoto Shibuya	**Plenary 6** Plant-microbe interactions II *Main Hall* Jian-Min Zhou Mary Beth Mudgett Martin Parniske Giles Oldroyd	**Concurrent 16** Recognition and signaling II *Main Hall* **Concurrent 17** Symbiosis II *Room A* **Concurrent 18** Endophytes and parasitic plants *Room D*
Lunch Break, *Banquet Halls SAKURA and SWAN*		
Concurrent 07 Effector proteins *Main Hall* **Concurrent 08** Plant-virus/viroid interactions *Room A* **Concurrent 09** Cell wall modification and resistance *Room D*	**Concurrent 13** Plant response *Main Hall* **Concurrent 14** Pathogenic bacteria/phytoplasma *Room A* **Concurrent 15** Systems biology *Room D*	**Concurrent 19** Biotechnology *Main Hall* **Concurrent 20** Genomics and evolution of virulence in pathogenic fungi and oomycetes *Room A* **Concurrent 21** Structural biology *Room D*
Coffee Break		**Coffee Break**
Concurrent 10 Plant hormones integrating defense response *Main Hall* **Concurrent 11** Crop protection *Room A* **Concurrent 12** Evolution of susceptibility and resistance *Room D*	**Excursion** 15:40 - 20:00	**Plenary 8** Plant-microbe interactions III *Main Hall* Thomas Lahaye Jens Stougaard Shou-Wei Ding Junji Takabayashi
Poster Session II (Even number) *Event Hall*		**Closing Ceremony** 18:00 - 18:30 *Main Hall*
		Congress Dinner 19:00 - 21:00 *Prince Hall (Grand Prince Hotel Kyoto)*

IS-MPMI XV CONGRESS HIGHLIGHTS

Sunday, July 29

Opening Lecture by Dr. Shizuo Akira and the Award Lecture

17:00 - 19:00 • Main Hall
Opening Lecture: Shizuo Akira will present the opening lecture titled “Innate immunity in mammals.” Akira contributes to a more comprehensive understanding of the dynamics of the immune system by employing not only traditional immunology experiments but also a variety of imaging and bioinformatics technologies.

Award Lecture: Eva Kondorosi and Adam Kondorosi, biologists at the Institut des Sciences du Végétal, were selected for the 2012 IS-MPMI Award for their innovative research in plant biology, particularly in the *Rhizobium*-legume symbiosis. Eva Kondorosi will accept the award and present the award lecture titled "Innate immunity effectors and virulence factors in symbiosis."

Welcome Reception

19:15 - 21:00 • Banquet Hall Swan
Join friends and colleagues for food, drinks, and conversation at the official welcome reception of the XV International Congress. This immediately follows the Opening Lecture and the Award Lecture.

Wednesday, August 1

Excursion

15:40 - 20:00
See the world heritage sites, Kiyomizu-dera and Gion Hanamikoji dori, on this 4-hour long sightseeing tour. **This event will take place come rain or shine.** ***Preregistration is required. Tickets are necessary to be admitted on the bus.***

Schedule: 16:00: ICC Kyoto
16:40: Kiyomizu-dera
(1-hour long guided tour and free time)
18:00: Gion Hanamikoji dori
(A view of the city from the window of the bus)
Stop by hotels in Kyoto
20:00: Grand Prince Hotel Kyoto

Thursday, August 2

Congress Dinner

19:00 - 21:00 • Prince Hall at the Grand Prince Hotel Kyoto
Get to know the researchers, professors, speakers, and suppliers who attend the congress! This year’s Congress Dinner includes a full-course meal with beverages and a variety of entertainment, including dancing. The Congress Dinner is the perfect place to mingle with attendees and their guests. ***Preregistration is required. Tickets are necessary to be admitted to the Congress Dinner.***

DAILY PROGRAM SCHEDULE AND SESSIONS *Pages 15-35*

All sessions take place in the Kyoto International Conference Center unless otherwise noted in the program schedule.

Sunday, July 29

10:00 - 20:00	Registration Open		Reception, Main Entrance
12:00 - 16:30	Board of Directors Meeting		Room H
14:00 - 17:30	Poster set-up (authors place posters in Event Hall)		Event Hall
12:00 - 14:00	**Special Workshop 1**	Imaging plant-microbe interactions	Room A
	Special Workshop 2	Induced susceptibility in plants	Room B-1
	Special Workshop 3	Powdery mildew	Room D
14:00 - 14:30	Break		
14:30 - 16:30	**Special Workshop 4**	Rice immunity and pathogens	Room A
	Special Workshop 5	Functional genomics of plant pathogenic bacteria	Room B-1
	Special Workshop 6	Proteomics	Room D
16:30 - 17:00	Break		
17:00 - 19:00	**Opening Remark**		Main Hall
	Opening Lecture	Dr. Shizuo Akira	Main Hall
	Award Lecture	Dr. Eva Kondorosi	Main Hall
19:15 - 21:00	**Welcome Reception**		Swan

SESSIONS – Sunday Afternoon

Special Workshop 1 - Imaging plant-microbe interactions
12:00 - 14:00; Room A
Co-Chairs: Andrea Genre, University of Turin, Italy
Noriko Inada, Nara Institute of Science and Technology, Japan
Daigo Takemoto, Nagoya University, Japan

12:00 **SW1-1. Pathogen-induced sugar transporters identified with the help of optical sensors.** L.Q. Chen. Carnegie Institute, USA
12:20 **SW1-2. Imaging of *Ustilago maydis* infection to maize.** S. Tanaka. Max Planck Institute for Terrestrial Microbiology, Germany
12:40 **SW1-3. Plants communicating with pathogens: membranes in motion and cellular defense.** S. Robatzek. The Sainsbury Laboratory, UK
13:00 **SW1-4. Imaging powdery mildew-plant interaction; manipulation of host cells by powdery mildew.** N. Inada. Nara Institute of Science and Technology, Japan
13:20 **SW1-5. Imaging of pathogenic and symbiotic fungi in culture and during infection.** D. Takemoto. Nagoya University, Japan
13:40 **SW1-6. Visualising perifungal membrane biogenesis in living arbuscular mycorrhizal roots.** A. Genre. University of Turin, Italy

Special Workshop 2 - Induced susceptibility in plants
12:00 - 14:00; Room B-1
Co-Chairs: Kazuya Akimitsu, Kagawa University, Japan
Gillian Turgeon, Cornell University, USA

12:00 **SW2-1. Induced susceptibility in citrus by *Alternaria* host-selective toxin.** K. Akimitsu. Kagawa University, Japan
12:20 **SW2-2. Comparative genome structure, secondary metabolite capacity and host-selective toxin production across *Cochliobolus* pathogens.** G. Turgeon. Cornell University, USA
12:40 **SW2-3. Plant cell wall is the first line of defense: a host-specific modulation for induced susceptibility.** K. Toyoda. Okayama University, Japan
13:00 **SW2-4. Tryptophan-derived metabolites in Mlo-mediated susceptibility of *Arabidopsis thaliana***

to *Golovinomyces orontii.* P. Bednarek. Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland

13:20 **SW2-5. Defining the composition and function(s) of the *Mycosphaerella graminicola* predicted protein secretome.** A. Amaral. Embrapa LabEx - Rothamsted Research, UK

13:40 **SW2-6. Investigation of the SnTox1-*Snn1* interaction: How is *Stagonospora nodorum* inducing susceptibility in wheat?** T. Friesen. USDA-ARS Northern Crop Science Laboratory, USA

Special Workshop 3 - Powdery mildew

12:00 - 14:00; Room D

Co-Chairs: Pietro Spanu, Imperial College London, UK
Hans Thordal-Christensen, University of Copenhagen, Denmark

12:00 **SW3-1. Arabidopsis BAX Inhibitor-1 co-immunoprecipitates with a cytochrome p450 monooxygenase, which is a susceptibility factor to powdery mildew.** R. Hückelhoven. Technische Universität München, Germany

12:20 **SW3-2. Identification of *Blumeria graminis* f.sp. *hordei* avirulence effectors using combination of a high- throughput functional screening and population genomics.** T. Maekawa. Max Planck Institute for Plant Breeding Research, Germany

12:40 **SW3-3. Study of the Pm3-mediated resistance response against *Blumeria graminis* f.sp. *tritici* in transgenic Arabidopsis plants.** T. Jordan. University of Zürich, Switzerland

13:00 **SW3-4. The structure and evolution of barley powdery mildew effector candidates.** C. Pedersen. University of Copenhagen, Denmark

13:20 **SW3-5. Arabidopsis mutants displaying aberrant localization of the PEN3 ABC transporter have altered responses to powdery mildew fungi.** W. Underwood. University of California, USA

13:40 **SW3-6. Arabidopsis powdery mildew effector proteins target highly connected host proteins and display virulence activity.** R.Wessling. Max Planck Institute for Plant Breeding Research, Germany

Special Workshop 4 - Rice immunity and pathogens

14:30 - 16:30; Room A

Chair: Guo-Liang Wang, Ohio State University, USA

14:30 **SW4-1. Molecular mechanism of the AvrPiz-t and Piz-t interaction" The *Magnaporthe oryzae* effector *AvrPiz-t* targets the ubiquitin-proteasome system for its avirulence and virulence activities in rice.** G.L. Wang. Ohio State University, USA

14:50 **SW4-2. Environmental impacts on rice immunity.** J. Leach. Colorado State University, USA

15:10 **SW4-3. Genetic studies of signaling pathways for innate immunity of rice.** A. Takahashi. National Institute of Agrobiological Sciences, Japan

15:30 **SW4-4. Spatio-temporal regulation of cell signaling during Magnaporthe pathogenesis.** N. Naqvi. Temasek Life Sciences Laboratory, Singapore

15:50 **SW4-5. Regulatory networks of Magnaporthe involved in rice infection.** M.H. Lebrun. CNRS-BayerCropScience, France

16:10 **SW4-6. Plant surface signals and appressorium morphogenesis in *Magnaporthe oryzae.*** JR. Xu. Purdue University, USA

Special Workshop 5 - Functional genomics of plant pathogenic bacteria

14:30 - 16:30; Room B-1

Co-Chairs: Alan Collmer, Cornell University, USA
Shinji Tsuyumu, Shizuoka University, Japan

14:30 **SW5-1. Genomics research on *Pectobacterium* and *Dickeya* species: Disease, ecology and diagnosis.** I. Toth. The James Hutton Institute, UK

14:50 **SW5-2. Molecular biological studies on phytoplasmal pathogenicity.** S. Namba. The University of Tokyo, Japan

15:10 **SW5-3. With and without a priori approaches to uncover pathogenicity determinants on the**

***Ralstonia solanacearum* genome.** N. Peters. UMR INRA-CNRS, France

15:30 **SW5-4. Bacterial genomics and the rise of microbial GWAS and reverse ecology.** D. Baltrus. University of Arizona, USA

15:50 **SW5-5. Exploring minimal functional repertoires of *Pseudomonas syringae* type III effectors.** A. Collmer. Cornell University, USA

16:10 **SW5-6. Genomic studies on regulatory mechanisms involved in bacterial plant pathology.** S. Tsuyumu. Shizuoka University, Japan

Special Workshop 6 - Proteomics

14:30 - 16:30; Room D

Co-Chairs: Alex Jones, The Sainsbury Laboratory, UK
Hirofumi Nakagami, RIKEN Plant Science Center, Japan

14:30 **Introduction**

14:35 **SW6-1. Dynamic changes in the plasma membrane proteome during plant immune signaling.** G. Coaker. University of California at Davis, USA

15:05 **SW6-2. Leaf oil bodies produce an anti-fungal compound actively in dying tissues.** T. Shimada. Kyoto University, Kyoto, Japan

15:15 **SW6-3. Identifying factors involved in pathogenicity of *Ralstonia solanacearum* strains at low temperatures using a proteomics approach.** A. Bocsanczy. University of Florida, USA

15:25 **SW6-4. Activity-based protein profiling: analyzing the effect of pathogenic nematodes on Arabidopsis roots.** S. Siddique. University of Bonn, Germany

15:35 **SW6-5. Phosphoproteomics approaches for signaling dissection.** H. Nakagami. RIKEN Plant Science Center, Japan

15:45 **SW6-6. Mechanism of CDPK function in local and systemic plant innate immune responses.** T. Romeis. Dahlem Centre of Plant Sciences, Germany

15:55 **SW6-7. The interaction proteome of the N NB-LRR immune receptor.** P. Cournoyer. Yale University, USA

16:05 **SW6-8. Targeted quantification of phosphorylation sites.** A. Jones. The Sainsbury Laboratory, UK

16:15 **Discussion**

SESSIONS – Sunday Evening

Opening and Award Lectures

17:00 - 19:00; Main Hall

Chair: Naoto Shibuya, Meiji University, Japan

Opening Lecture

OL-1. Innate immunity in mammals. S. Akira[1]. [1]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Japan

Award Lecture

AL-1. Innate immunity effectors and virulence factors in symbiosis. E. Kondorosi[1,2], A. Kondorosi[1]. [1]Institut des Sciences du Vegetal, CNRS, 91198 Gif sur Yvette, France, [2]Biological Research Centre of the Hungarian Academy of Sciences, Hungary

Monday, July 30

7:30 - 17:00	Registration		Reception, Main Entrance
8:30 - 20:30	Exhibits Open and Poster viewing		Event Hall
8:30 - 10:00	**Plenary 1**	Plant signaling I	Main Hall
10:00 - 10:20	Coffee Break		
10:20 - 12:20	**Plenary 2**	Pathogen	Main Hall
12:20 - 13:30	Lunch Break		Sakura and Swan
	Poster Viewing and Exhibits Open		Event Hall
13:30 - 15:30	**Concurrent 01**	Recognition and signaling I	Main Hall
	Concurrent 02	Symbiosis I	Room A
	Concurrent 03	Pathogenic fungi	Room D
15:30 - 15:50	Coffee Break		
15:50 - 17:50	**Concurrent 04**	Plant-oomycete / fungal interactions	Main Hall
	Concurrent 05	Biocontrol interactions	Room A
	Concurrent 06	Plant-nematode / insect interactions	Room D
18:00 - 20:30	**Poster Session I**		Event Hall
	Poster Viewing and Exhibits Open 18:00 - 20:00 *Odd-numbered poster authors present*		

SESSIONS – Monday Morning

Plenary 1 - Plant signaling I
8:30 - 10:00; Main Hall
Chair: Cyril Zipfel, The Sainsbury Laboratory, UK

8:30 **PL1-1. Regulation of surface immune receptor complex activity.** C. Zipfel[1]. [1]The Sainsbury Laboratory

9:00 **PL1-2. The role of Fusarium effectors in NLR-mediated innate immunity.** F. Takken[1], L. Ma[1], P. Houterman[1], F. Gawehns[1], M. de Sain[1], F. Sillo[1], B. Cornelissen[1], M. Rep[1]. [1]Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

9:30 **PL1-3. Defensome in rice innate immunity.** K. Shimamoto[1], A. Akamatsu[1], S. Hamada[1], Y. Kawano[1]. [1]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, Japan

Plenary 2 - Pathogen
10:20 - 12:20; Main Hall
Chair: Sophien Kamoun, The Sainsbury Laboratory, UK

10:20 **PL2-1. Defining the core *Arabidopsis thaliana* root microbiome.** D. S. Lundberg[1], S. L. Lebeis[1], S. H. Paredes[1], S. Yourstone[1], S. G. Tringe[2], J. Dangl[1,3]. [1]University of North Carolina at Chapel Hill, [2]DOE Joint Genome Institute, [3]Howard Hughes Medical Institute

10:50 **PL2-2. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota.** B. Davide[1], M. Rott[1], K. Schlaeppi[1], E. Ver Loren van Themaat[1], N. Ahmadinejad[1], F. Assenza[1], T. Eickhorst[2], P. Schulze-Lefert[1]. [1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, [2]Institute of Soil Science, University of Bremen

11:20 **PL2-3. Oomycetes, effectors, and all that jazz.** S. Kamoun[1], T. O. Bozkurt[1], L. M. Cano[1], A. Chaparro-Garcia[1], S. Dong[1], S. R. F. King[2], K. Kowitwanich[1], V. Nekrasov[1], M. Pais[1], S. Raffaele[1], D. G. O. Saunders[1], S. Schornack[1], J. Win[1], K. Yoshida[1], M. J. Banfield[2]. [1]The Sainsbury Laboratory, [2]Dept. of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

11:50 **PL2-4. Systems biology initiatives for the rice blast fungus.** Y.-H. Lee[1]. [1]Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, Korea

SESSIONS – Monday Afternoon

Concurrent 01 - Recognition and signaling I
13:30 - 15:30; Main Hall
Co-Chairs: David Guttman, University of Toronto, Canada
Thorsten Nuernberger, University of Tuebingen, Germany

13:30 **CS01-1. Patterns and receptors in Arabidopsis immunity.** T. Nuernberger[1]. [1]University of Tuebingen, Center for Plant Molecular Biology

13:50 **CS01-2. OsRLCK2 targeted by *Xanthomonas* Xoo1488 effector regulates MAP kinase cascade activated by OsCERK1-mediated recognition of chitin in rice.** K. Yamaguchi[1], K. Yamada[1], K. Ishikawa[1], M. Kishi-Kaboshi[2], A. Takahashi[2], S. Tsuge[3], K. Ichimura[4], H. Yoshioka[5], K. Shimamoto[6], T. Kawasaki[1]. [1]Graduate School of Agriculture, Kinki University, [2]Division of Plant Sciences, National Institute of Agrobiological Sciences, [3]Graduate School of Agriculture, Kyoto Prefectural University, [4]Graduate School of Agriculture, Kagawa University, [5]Graduate School of Bioagricultural Sciences, Nagoya University, [6]Graduate School of Biological Science, Nara Institute of Science and Technology

14:10 **CS01-3. Identification of a receptor-like kinase (RLK) required for functionality of receptor-like proteins (RLPs) involved in pathogen resistance of tomato.** M. H. A. J. Joosten[1,4], P. E. J. Smit[1], A. Abd-El-Haliem[1], A. Kombrink[1], R. de Jonge[1], J. H. G. Cordewener[2,4], A. H. P. America[2,4], J. Sklenar[3], A. M. E. Jones[3], S. Robatzek[3], G. C. M. van den Berg[1], B. P. H. J. Thomma[1,4], W. I. L. Tameling[1], T. W. H. Liebrand[1,4]. [1]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands, [2]Plant Research International, Wageningen UR, Wageningen, The Netherlands., [3]The Sainsbury Laboratory, Norwich Research Park, United Kingdom, [4]Centre for BioSystems Genomics, 6700 AB Wageningen, The Netherlands.

14:30 **CS01-4. Identification of innate immunity elicitors using molecular signatures of natural selection.** H. C. McCann[1], H. Nahal[2], S. Thakur[1], D. S. Guttman[1,2]. [1]Department of Cell & Systems Biology, University of Toronto, Toronto Canada, [2]Centre for Genome Evolution & Function, University of Toronto, Toronto Canada

14:50 **CS01-5. Bacterial effector manipulates JAZ transcription repressors of jasmonate signaling to facilitate bacterial infection.** S. Jiang[1], J. Yao[3], H. Zhou[1,2,5], K.-W. Ma[1], S.-Y. He[3,4], W. Ma[1,2,5]. [1]Department of Plant Pathology and Microbiology, University of California, Riverside, California, USA, [2]Institute of Integrative Genomics, University of California, Riverside, CA 92521, USA, [3]DOE Plant Research Laboratory, Michigan State University, East lancing, MI 48824, USA, [4]Department of Plant Biology, Michigan State University, East lancing, MI 48824, USA, [5]Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA

15:10 **CS01-6. Lectin receptor kinases as modulators of the Arabidopsis innate immunity response.** L. Zimmerli[1], M. Desclos-Theveniau[1], P. Singh[1]. [1]Institute of Plant Biology and Department of Life Science, National Taiwan University, Taipei, Taiwan

Concurrent 02 - Symbiosis I
13:30 - 15:30; Room A
Co-Chairs: Masayoshi Kawaguchi, National Institute for Basic Biology, Japan
Sharon Long, Stanford University, USA

13:30 **CS02-1. *inhospitable*, a novel rice mutant abolishes hyphopodia formation by arbuscular mycorrhizal fungi.** C. Gutjahr[1,2], M. Riemann[3,4], K. Haga[5], M. Takano[3], M. Iino[5], P. Nick[4], U. Paszkowski[2]. [1]Institute of Genetics, Faculty of Biology, University of Munich (LMU), Germany, [2]Department of Plant Molecular Biology, University of Lausanne, 1015 Lausanne, Switzerland, [3]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan, [4]Botanical Institute 1, University of Karlsruhe, 76128 Karlsruhe, Germany, [5]Botanical Gardens, Graduate School of Science, Osaka City University, Kisaichi, Katano-shi, Osaka 476-0004, Japan

13:50 **CS02-2. HAR1, KLAVIER and TOO MUCH LOVE mediate CLE peptide signaling in long-distance control of nodulation.** M. Kawaguchi[1]. [1]Division of Symbiotic Systems, National Institute for Basic Biology, Okazaki, Japan

14:10 **CS02-3. Activation of the host symbiosis signaling by rhizobial type III secretion system.** S. Okazaki[1], T. Kaneko[2], S. Sato[3], K. Saeki[4]. [1]Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan, [2]Kyoto Sangyo University, [3]Kazusa DNA Research

Institute, [4]Nara Women's University

14:30 **CS02-4. Studies on putative type III-secreted effector proteins containing a self-cleavable DUF1521 domain.** J. Schirrmeister[1], L. Flor[1], S. Zocher[1], M. Hoppe[1], A.-K. Hoffmeister[1], M. Gottfert[1], S. Zehner[1]. [1]Institute of Genetics, Department of Biology, Dresden University of Technology, Dresden, Federal Republic of Germany

14:50 **CS02-5. Zwitterionic membrane lipids phosphatidylethanolamine and phosphatidylcholine affect transcription and physiology of *Sinorhizobium meliloti* in different ways.** O. Geiger[1], D. B. Medeot[1], D. Vera-Cruz[1], D. X. Sahonero-Canavesi[1], S. Weidner[2], A. Puehler[2], I. M. Lopez-Lara[1], C. Sohlenkamp[1]. [1]Centro de Ciencias Genomicas, Universidad Nacional Autonoma de Mexico, [2]Institut fuer Genomforschung und Systembiologie, Centrum fuer Biotechnologie, Universitaet Biehlefeld

15:10 **CS02-6. *Sinorhizobium meliloti* ECF sigma factors are required for symbiosis on *Medicago sativa* and *M. truncatula*.** S. R. Long[1], M. E. Diodati[1], R. Fisher[1]. [1]Department of Biology, Stanford University, Stanford CA, USA

Monday

Concurrent 03 - Pathogenic fungi

13:30 - 15:30; Room D

Co-Chairs: Yasuyuki Kubo, Kyoto Prefectural University, Japan
You-Liang Peng, China Agricultural University, China

13:30 **CS03-1. A novel component of the Prp19-associated complex is essential to safeguarding efficient intron splicing of pathogenicity genes in the rice blast fungus.** Y.-L. Peng[1], J. Yang[1], W. Wang[1], L. Kong[1], X. Chen[1], W. Zhao[1], D. Wang[1], M. Xue[1], J. Sun[1], X. Zhou[2], Y. Zhang[3], J. Liu[3], R. Wang[1], X. Xu[1], Y. Xing[1], J.-R. Xu[2]. [1]State Key Laboratory of Agrobiotechnology and Department of Plant Pathology, China Agricultural University, Beijing 100193, China, [2]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA, [3]Department of Plant Pathology, China Agricultural University, Beijing 100193, China

13:50 **CS03-2. ChTn1, a Tc1-mariner transposable element of *Cochliobolus heterostrophus* is regulated by intron retention.** M. V. Queiroz[1], B. G. Turgeon[2]. [1]Department of Microbiology, Federal University of Vicosa, Vicosa, Minas Gerais, Brazil, [2]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA

14:10 **CS03-3. Roles of histone lysine methyltransferases in the pathogenicity of *Magnaporthe oryzae*.** K. T. M. Pham[1], B. Vu[1], Q. Nguyen[1], H. Nakayashiki[1]. [1]Graduate School of Agricultural Sciences, Kobe University, Kobe, Japan

14:30 **CS03-4. A refinement of the predicted secretome for the wheat leaf pathogen *Mycosphaerella graminicola*.** A. Amaral[1,2], J. Antoniw[2], J. Rudd[2], K. Hammond-Kosack[2]. [1]Embrapa LabEx Europe, [2]Rothamsted Research

14:50 **CS03-5. Septin-mediated plant cell invasion by the rice blast fungus *Magnaporthe oryzae*.** Y. F. Dagdas[1], K. Yoshino[1], G. Dagdas[1], L. Ryder[1], E. Bielska[1], G. Steinberg[1], N. Tlabot[1]. [1]School of Biological Sciences, University of Exeter, Exeter, UK

15:10 **CS03-6. Pathogenesis and infection related morphogenesis of *Colletotrichum orbiculare*.** Y. Kubo[1]. [1]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan

Concurrent 04 - Plant-oomycete / fungal interactions

15:50 - 17:50; Main Hall

Co-Chairs: Yoshitaka Takano, Kyoto University, Japan
Barbara Valent, Kansas State University, USA

15:50 **CS04-1. Nonhost interactions between *Arabidopsis* and anthracnose fungi.** Y. Takano[1], K. Hiruma[1]. [1]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

16:10 **CS04-2. RAC/ROP G-protein interacting proteins of barley are involved in microtubule organization and basal resistance to penetration by the barley powdery mildew fungus.** R. Huckelhoven[1], T. Reiner[1], C. Hoefle[1]. [1]Technical University of Munich, TUM-Phytopathology

16:30 **CS04-3. Mechanisms of secretion and delivery of rice blast effector proteins into live rice cells.** B. Valent[1], M. C. Giraldo[1], M. Yi[1], C.-H. Khang[1,2], M. Dalby[1], Y. Dagdas[3], Y. K. Gupta[3], N. J.

Talbot[3], M. Farman[4]. [1]Department of Plant Pathology, Kansas State University, Manhattan, Kansas, [2]Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA, [3]School of Biosciences, University of Exeter, Exeter EX4 4 QD, UK, [4]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, 40546, USA

16:50 **CS04-4. *Phytophthora* effectors facilitate infection by suppressing host RNA silencing.** W. Ma[1,3], Y. Qiao[1], L. Liu[2], J. Wong[1], C. Flores[1], H. Judelson[1,3], X. Chen[2,3]. [1]Department of Plant Pathology and Microbiology, [2]Department of Botany and Plant Sciences, University of California Riverside, [3]Institute for Integrative Genome Biology, University of California Riverside

17:10 **CS04-5. Isolation and functional characterization of the host targets of *Phytophthora infestans* RXLR effector Avr-chc1.** A. Abd-El-Haliem[1], J. Win[2], S. Schornack[2], J. Sklenar[2], S. Kamoun[2], V. Vleeshouwers[1], Y. Bai[1], J. Vossen[1]. [1]Laboratory of Plant Breeding, Wageningen University, The Netherlands, [2]Sainsbury Laboratory; Norwich, NR4 7UH, United Kingdom

17:30 **CS04-6. Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species.** I. Chuma[1], C. Isobe[1], Y. Hotta[1], K. Ibaragi[1], N. Futamata[1], M. Kusaba[2], K. Yoshida[3], R. Terauchi[3], Y. Fujita[4], H. Nakayashiki[1], B. Valent[5], Y. Tosa[1]. [1]Graduate School of Agricultural Sciences, Kobe University, Kobe, Japan, [2]Faculty of Agriculture, Saga University, Saga, Japan, [3]Research group of Genetics and Genomics, Iwate Biotechnology Research Center, Kitakami, Japan, [4]College of Bioresource Sciences, Nihon University, Kanagawa, Japan, [5]Department of Plant Pathology, Kansas State University, Manhattan, Kansas, United States of America

Concurrent 05 - Biocontrol interactions

15:50 - 17:50; Room A

Co-Chairs: Hideo Nakashita, Tokyo University of Agriculture, Japan, RIKEN, Japan
Barry Scott, Molecular BioSciences, New Zealand

15:50 **CS05-1. Regulation of bioprotective metabolite biosynthesis in the grass symbiont *Epichloe festucae*.** D. B. Scott[1], T. Chujo[1], D. Barry[1]. [1]Molecular BioSciences

16:10 **CS05-2. Effect of colonization of endophytic bacteria on rice.** H. Nakashita[1,3], T. Isawa[2,3], M. Yasuda[2], M. Kusajima[1,3], J. Hirayama[2,3], K. Minamisawa[4], S. Shinozaki[2,3]. [1]Department of Applied Biology and Chemistry, Tokyo University of Agriculture, [2]Research and Development Center, Mayekawa MFG. CO., LTD., [3]RIKEN Innovation Center, RIKEN, [4]Graduate School of Life Sciences, Tohoku University.

16:30 **CS05-3. ppGpp controlled by the Gac/Rsm regulatory pathway sustains biocontrol activity in *Pseudomonas fluorescens* CHA0.** K. Takeuchi[1], K. Yamada[2], D. Haas[3]. [1]National Institute of Agrobiological Sciences, [2]University of Tsukuba, [3]Université de Lausanne

16:50 **CS05-4. Role of the root-specific transcription factor MYB72 in rhizobacteria-induced systemic resistance.** C. Zamioudis[1], P. A. H. M. Bakker[1], C. M. J. Pieterse[1]. [1]Utrecht University

17:10 **CS05-5. *Paenibacillus polymyxa* M-1, a plant growth promoting rhizobacterium, is capable of colonizing the roots of wheat.** Q. Wang[1], B. Niu[1], R. Borriss[2], X. Chen[2], J. Vater[3], A. Hartmann[4], Y. Li[1], W. Bleiss[5]. [1]Department of Plant Pathology, China Agricultural University, P.R. China, [2]Institut für Biologie/Bakteriengenetik, Humboldt Universität Berlin, Berlin, Germany, [3]Institut für Chemie, Technische Universität Berlin, Berlin, Germany, [4]Department Microbe-Plant Interactions, Helmholtz Zentrum München, Germany, [5]Institut für Biologie/Molekulare Parasitologie, Humboldt Universität Berlin, Berlin, Germany

17:30 **CS05-6. Loss of virulence in the phytopathogen *Ralstonia solanacearum* through infection by φRSM filamentous phages.** T. Yamada[1], H. S. Addy[1], T. Kawasaki[1], M. Fujie[1]. [1]Graduate School of Advanced Sciences of Matter. Hiroshima University, Higashi-Hiroshima, Japan

Concurrent 06 - Plant-nematode / insect interactions

15:50 - 17:50; Room D

Co-Chairs: Pierre Abad, UMR ISA INRA, France
Derek Goto, Hokkaido University, Japan

15:50 **CS06-1. Finding new candidate parasitism genes in plant parasitic nematodes: an evolutionary and comparative genomics approach.** P. Abad[1], L. Perfus-Barbeoch[1], A. Campan-

Fournier[1], M.-J. Arguel[1], M. Da Rocha[1], M.-N. Rosso[1], E. G. J. Danchin[1]. [1]UMR ISA INRA 1355-UNSA-CNRS 7254, Institut Sophia Agrobiotech, Sophia Antipolis, France

16:10 **CS06-2. Mining the active proteome of nematode-induced feeding cells in roots of *Arabidopsis thaliana*.** S. Siddique[1], M. Huetten[1], M. Geukes[1], J. Misas-Villamil[2], R. van der Hoorn[2], F. M. W. Grundler[1]. [1]INRES, Department of Molecular Phytomedicine, University of Bonn, [2]Plant Chemetics lab, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

16:30 **CS06-3. Interaction between root-knot nematodes and plant signaling networks during parasitic invasion.** S. Hayashi[1], N. Souda[2], T. Ezawa[3], M. Kawaguchi[4], E. Asamizu[2], D. Goto[3]. [1]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan, [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan, [3]Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan, [4]Division of Symbiotic Systems, National Institute for Basic Biology, Okazaki, Japan

16:50 **CS06-4. Tritrophic interactions among thrips, tospovirus and Arabidopsis.** H. Abe[1], Y. Tomitaka[2], T. Shimoda[2], S. Seo[3], T. Sakurai[4], S. Kugimiya[5], S. Tsuda[2], M. Kobayashi[1]. [1]RIKEN BioResource Center, [2]National Agricultural Research Center, [3]National Institute of Agrobiological Sciences, [4]National Agricultural Research Center for Tohoku Region, [5]National Institute for Agro-Environmental Sciences

17:10 **CS06-5. Rewiring of the jasmonate signaling pathway in Arabidopsis during insect herbivory.** S. C. M. Van Wees[1], A. Verhage[1], C. M. J. Pieterse[1]. [1]Plant-Microbe Interactions, Utrecht University, The Netherlands

17:30 **CS06-6. Involvement of MAP kinase cascade and NO in plant immune response to *Henosepilachna vigintioctopunctata*.** N. Senga[1], Y. Sato[2], T. Niimi[3], H. Yoshioka[1]. [1]Defense in Plant-Pathogen Interactions, Graduated School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [2]Plant Genetics and Breeding, Graduated School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [3]Sericulture and Entomoresources, Graduated School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

Tuesday, July 31

8:00 - 17:00	Registration		Reception, Main Entrance
8:30 - 20:30	Poster viewing		Event Hall
8:30 - 10:00	**Plenary 3**	Plant immunity I	Main Hall
10:00 - 10:20	Coffee Break		
10:20 - 12:20	**Plenary 4**	Plant-microbe interactions I	Main Hall
12:20 - 13:30	Lunch Break		Sakura and Swan
	Poster Viewing and Exhibits Open		Event Hall
13:30 - 15:30	**Concurrent 07**	Effector proteins	Main Hall
	Concurrent 08	Plant-virus / viroid interactions	Room A
	Concurrent 09	Cell wall modification and resistance	Room D
15:30 - 15:50	Coffee Break		
15:50 - 17:50	**Concurrent 10**	Plant hormones integrating defense response	Main Hall
	Concurrent 11	Crop protection	Room A
	Concurrent 12	Evolution of susceptibility and resistance	Room D
18:00 - 20:30	**Poster Session II**		Event Hall
	Poster Viewing and Exhibits Open 18:00 - 20:00 *Even-numbered poster authors present*		

SESSIONS – Tuesday Morning

Plenary 3 - Plant immunity I

8:30 - 10:00; Main Hall

Chair: Jane Parker, Max-Planck Institute for Plant Breeding Research, Germany

8:30 **PL3-1. Partitioning of effector-triggered immune outputs in plant cells.** J. E. Parker[1], S. Blanvillain-Baufume[1], K. Heidrich[1], N. Peine[1], L. Deslandes[2], C. Tasset[2], S. Rietz[1], S. Wagner[3], J. Stuttmann[1], K. Niefind[3]. [1]Dept. Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Cologne, Germany, [2]CNRS/INRA Laboratoire des Interactions Plantes-Microorganismes, Castanet-Tolosan, France, [3]Institute of Biochemistry, University of Cologne, Cologne, Germany

9:00 **PL3-2. How oomycete pathogens of Arabidopsis cause or fail to cause disease.** J. Jones[1], E. Kemen[1], K. Sohn[1], L. Wirthmueller[1], S. Asai[1], M.-C. Caillaud[1], A. Kemen[1], A. Robert-Seilaniantz[1], S. Saucet[1], O. Furzer[1]. [1]Sainsbury Lab

9:30 **PL3-3. Messages from powdery mildew DNA: how interplay with the host moulds the pathogen genomes.** P. D. Spanu[1]. [1]Imperial College London

Plenary 4 - Plant-microbe interactions I

10:20 - 12:20; Main Hall

Chair: Maria Harrison, Boyce Thompson Institute for Plant Research, USA

10:20 **PL4-1. Reprogramming root cells for arbuscular mycorrhizal (AM) symbiosis.** M. J. Harrison[1]. [1]Boyce Thompson Institute for Plant Research

10:50 **PL4-2. Evolution of Rhizobium nodule symbiosis.** T. Bisseling[1,2], E. Fedorova[1], E. Limpens[1], R. Geurts[1]. [1]Wageningen University, graduate school Experimental Plant Sciences, Wageningen, The Netherlands, [2]King Saud University, Riyadh, Saudi Arabia

11:20 **PL4-3. What did we learn from the MOSes?** X. Li[1]. [1]Michael Smith Laboratories/Botany, University of British Columbia, Vancouver, BC, Canada

11:50 **PL4-4. Chitin receptors in plant immunity.** N. Shibuya[1], H. Kaku[1], T. Shinya[1], T. Shimizu[1], T. Nakagawa[1], N. Motoyama[1]. [1]Department of Life Sciences, Meiji University

SESSIONS – Tuesday Afternoon

Concurrent 07 - Effector proteins
13:30 - 15:30; Main Hall
Co-Chairs: Jean T. Greenberg, The University of Chicago, USA
Ryohei Terauchi, Iwate Biotechnology Research Center, Japan

13:30 **CS07-1. *Pseudomonas syringae* type III effectors.** J. Greenberg[1], J. Lee[1], Y. Kang[1], J. Jelenska[1], T. Wroblewski[2], R. W. Michelmore[2]. [1]The University of Chicago, [2]University of California, Davis

13:50 **CS07-2. The *Xanthomonas oryzae* pv. *oryzae* type III effector XopR alters ethylene perception and signal transduction pathway post MAMPs treatment, suppresses plant innate immunity in *Arabidopsis thaliana*.** C. Akimoto-Tomiyama[1], A. Furutani[2], S. Tsuge[3], H. Ochiai[1]. [1]Plant-Microbe Interaction Research Unit, Division of Plant Sciences, National Institute of Agrobiological Sciences, Tsukuba, Japan, [2]Gene Research Center, Ibaraki University, Inashiki, Japan, [3]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto Prefectural University, Kyoto, Japan

14:10 **CS07-3. The *Pseudomonas syringae* type III effector HopD1 targets the ER-localized Arabidopsis transcription factor NTL9 and blocks effector-triggered immunity.** A. Block[1], T. Toruno[1], J. R. Alfano[1]. [1]Center for Plant Science Innovation and the Department of Plant Pathology The University of Nebraska, Lincoln, Nebraska 68588 USA

14:30 **CS07-4. Toward understanding *Magnaporthe oryzae* effector functions.** R. Terauchi[1]. [1]Iwate Biotechnology Research Center, Iwate, Japan

14:50 **CS07-5. High resolution crystal structure of *Cladosporium fulvum* LysM effector Ecp6.** A. Sanchez Vallet[1], R. S. B. Saleem Batcha[2], A. Kombrink[1], D. J. Valkenburg[1], J. Mesters[2], B. P. H. J. Thomma[1]. [1]Department of Phytopathology, Wageningen University, Wageningen, The Netherlands, [2]Institute of Biochemistry, University of Lubeck, D-23538 Lubeck, Germany

15:10 **CS07-6. Effectors secreted by plant pathogenic oomycetes as molecular probes to understand focal immune responses at pathogen penetration sites.** T. O. Bozkurt[1], S. Schornack[1], S. Raffaele[1], S. Kamoun[1]. [1]The Sainsbury Laboratory

Concurrent 08 - Plant-virus / viroid interactions
13:30 - 15:30; Room A
Co-Chairs: Masayuki Ishikawa, National Institute of Agrobiological Sciences, Japan
Na-Sheng Lin, Academia Sinica, Taiwan

13:30 **CS08-1. Microtubule (+)-end-associated protein interacting with potyviral helper component proteinase.** T. Haikonen[1], M.-L. Rajamaki[1], J. P. T. Valkonen[1]. [1]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

13:50 **CS08-2. *Tobacco mosaic virus* movement protein co-targets to plasmodesmata with virus-induced host β-1,3-glucanases.** B. L. Epel[1], R. Zavaliev[1], A. Levy[1]. [1]Department of Molecular Biology and Ecology of Plants

14:10 **CS08-3. Replication-independent long-distance trafficking of *Bamboo mosaic virus* satellite RNA.** N.-S. Lin[1]. [1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

14:30 **CS08-4. Transgene viral siRNA profile and its effect on cucurbit viral resistance.** A. Gal-On[1], D. Leibman[1]. [1]Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel

14:50 **CS08-5. The single dominant resistance gene *Tsw* is triggered by a functional RNA silencing suppressor protein of the *Tomato spotted wilt virus*.** D. de Ronde[1], P. Butterbach[1], D. Lohuis[1], M. Hedil[1], J. W. M. van Lent[1], R. Kormelink[1]. [1]Laboratory of Virology, Wageningen University, Wageningen, the Netherlands

15:10 **CS08-6. Genetic, biochemical, and structural studies about interactions between *Tomato mosaic virus* and the resistance gene *Tm-1*.** K. Ishibashi[1], S. Miyashita[1,2], M. Kato[1], Y. Kezuka[3], E. Katoh[1], M. Ishikawa[1]. [1]Plant-Microbe Interactions Research Unit, National Institute of Agrobiological Sciences, Tsukuba, Japan, [2]Japan Science and Technology Agency, Precursory Research for Embryonic Science and Technology, Kawaguchi, Japan, [3]Depertment of Structural Biology, School of Pharmacy, Iwate Medical University, Yahaba, Iwate, Japan

Concurrent 09 - Cell wall modification and resistance
13:30 - 15:30; Room D
Co-Chairs: Giulia De Lorenzo, Sapienza Universita' di Roma, Italy
Antonio Molina, Universidad Politecnica de Madrid, Spain

13:30 **CS09-1. Oligogalacturonides alert the plant immune system to cell wall damage.** G. De Lorenzo[1]. [1]Dip. Biologia e Biotecnologie C. Darwin, Sapienza Universita' di Roma, Roma, Italy

13:50 **CS09-2. Uncoupling resistance to pathogens from tradeoffs by remodeling Arabidopsis cell wall.** A. Molina[1], E. Miedes[1], M. P. Riviere[1], A. Sanchez-Vallet[1], C. Sanchez-Rodriguez[1], M. Delgado[1], L. Jorda[1], N. Denance[2], P. Ranocha[2], X. Bartel[3], Y. Marco[3], D. Goffner[2]. [1]Centro de Biotecnologia y Genomica de Plantas (UPM-INIA), Departamento Biotecnologia-UPM, Universidad Politecnica de Madrid, Madrid, Spain, [2]Unite Mixte de Recherche Centre Nacional de la Recherche Scientifique Univ Toulouse III, Pole de Biotechnologie Vegetale, BP 42617 Auzeville 24, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE, [3]Laboratoire de Interactions Plantes-Microorganismes, Centre Nacional de la Recherche Scientifique Instiut National de la Recherche Agronomique, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE

14:10 **CS09-3. Cell wall acetylation plays a pivotal role in the cuticle assembly and susceptibility to nectrotic fungal pathogen *Botrytis cinerea*.** M. Nafisi[1,2], M. Stranne[1,2], D. Silvestro[1,2], Y. Manabe[3], H. V. Scheller[3], M. Burrow[1,2], C. Nawrath[4], H. J. Martens[1], Y. Sakuragi[1,2]. [1]Department of Plant Biology and Biotechnology, [2]VKR Research Centre Pro-Active Plants, [3]DOE Joint Bioenergy Institute, California, USA, [4]Département de Biologie Moléculaire Végétale, Université de Lausanne, Switzerland

14:30 **CS09-4. Poly(ADP-ribosyl)ation plays an essential role in pathogen-induced cell wall reinforcement.** B. D. Keppler[1], A. G. Briggs[1], J. Song[2], A. F. Bent[2]. [1]Program in Cellular and Molecular Biology, University of Wisconsin, Madison, WI, [2]Department of Plant Pathology, University of Wisconsin, Madison, WI

14:50 **CS09-5. Reduced carbohydrate availability and altered pectin composition in Arabidopsis enhance susceptibility towards *Colletotrichum higginsianum*.** L. Voll[1], T. Engelsdorf[1], R. Horst[1], R. Proels[2], M. Proeschel[1], C. Will[1], J. Hofmann[1], R. Hueckelhoven[2]. [1]Division of Biochemistry, FAU Erlangen-Nuremberg, Erlangen, Germany, [2]Technical University Munich, Division of Phytopathology, Freising-Weihenstephan, Germany

15:10 **CS09-6. Links between the cell wall and powdery mildew disease resistance in Arabidopsis.** C. Cherk[1,2], Y. Verhertbruggen[3], H. Szemenyei[1,2], B. R. Dotson[1,2], C. Somerville[1,2], H. V. Scheller[1,3], S. Somerville[1,2]. [1]Department of Plant and Microbial Biology, UC Berkeley, [2]Energy Biosciences Institute, UC Berkeley, [3]Joint Bioenergy Institute and Physical Biosciences Division, Lawrence Berkeley National Laboratory

Concurrent 10 - Plant hormones integrating defense response
15:50 - 17:50; Main Hall
Co-Chairs: Xinnian Dong, Duke University, USA
Jane Glazebrook, University of Minnesota, USA

15:50 **CS10-1. Roles of CBP60 proteins in the plant defense network.** J. Glazebrook[1], L. Wang[1], W. Truman[1], S. Sreekanta[1]. [1]Department of Plant Biology, University of Minnesota

16:10 **CS10-2. Dynamic regulation of plant immune response.** X. Dong[1]. [1]Department of Biology, Duke University, Durham, North Carolina

16:30 **CS10-3. Rice WRKY45 plays a key role in priming of diterpenoid phytoalexin biosynthesis through the salicylic acid signaling pathway.** A. Akagi[1], S. Fukushima[1], K. Okada[2], C.-J. Jiang[1], R. Yoshida[3], M. Shimono[4], S. Sugano[1], H. Yamane[5], H. Takatsuji[1]. [1]Disease Resistant Crops Research Unit, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, [2]Biotechnology Research Center,The University of Tokyo, Tokyo, Japan, [3]Faculty of Agriculture, Kagoshima University, Kagoshima, Japan, [4]Department of Plant Pathology, Michigan State University, Michigan, Japan, [5]Department of Biosciences, Teikyo University, Tochigi, Japan

16:50 **CS10-4. Hormonal modulation of plant immunity.** C. M. J. Pieterse[1], D. Van der Does[1], A. Leon-Reyes[1], J. Memelink[2], S. C. M. Van Wees[1]. [1]Utrecht University, [2]Leiden University

17:10 **CS10-5. Brassinosteroids antagonize gibberellin- and salicylate-mediated root immunity in rice.** D. De Vleesschauwer[1], E. Van Buyten[1], K. Satoh[2], J. Balidion[3], R. Mauleon[4], I.-R. Choi[3], C. Vera-Cruz[3], S. Kikuchi[2], M. Hofte[1]. [1]Lab of Phytopathology, Ghent University, Ghent, Belgium,

[2]Plant Genome Research Unit, Agrogenomics Research Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, [3]Plant Breeding, Genetics, and Biotechnology Division, International Rice Research Institute, Metro Manila, Philippines, [4]Crop Research Informatics Laboratory, International Rice Research Institute, Metro Manila, Philippines

17:30 **CS10-6. Tyrosine sulfated peptide receptors PSKR1 and PSY1R modulate *Arabidopsis* immunity.** S. L. Mosher[1], H. Seybold[1], P. Rodriguez[1], M. Stahl[2], M. Wierzba[3], K. Davies[3], S. Morillo[3], S. Dayaratne[3], F. E. Tax[3], B. Kemmerling[1]. [1]ZMBP Plant Biochemistry, Eberhard-Karls-University Tuebingen, Tuebingen, Germany, [2]ZMBP Analytics, Eberhard-Karls-University Tuebingen, Tuebingen, Germany, [3]Department of Molecular and Cellular Biology, University of Arizona, Tucson, USA

Concurrent 11 - Crop protection

15:50 - 17:50; Room A

Co-Chairs: Pierre J. G. M. de Wit, Wageningen University, Netherlands
Tina Jordan, University of Zurich, Switzerland

15:50 **CS11-1. The wheat Mla homologue TmMla1 exhibits an evolutionary conserved function against powdery mildew in both wheat and barley.** T. Jordan[1], S. Milani[1], S. Seeholzer[1], A. Toeller[2], S. Schwizer[1], I. E. Somssich[2], B. Keller[1]. [1]Institute of Plant Biology, University of Zurich, Switzerland, [2]Max-Planck-Institute for Plant Breeding, Department of Plant-Microbe Interactions, Carl-von-Linne Weg 10, D-50829 Koeln, Germany

16:10 **CS11-2. Allele pyramiding of the wheat powdery mildew resistance gene *Pm3*: A strategy for more durable resistance?** D. Stirnweis[1], S. Brunner[1], T. Jordan[1], B. Keller[1]. [1]Institute of Plant Biology, University of Zurich, Zurich, Switzerland

16:30 **CS11-3. The genome of the fungus *Cladosporium fulvum* suggests an ancestral host jump to tomato.** P. J. G. M. De Wit[1], A. van der Burgt[1], B. Okmen[1], I. Stergiopoulos[1,2], A. Bahkali[3], H. Beenen[1], P. Chettri[4], Y. Guo[4], S. Kabir[4], M. Karimi Jashni[1], R. Mehrabi[1], J. Collemare[1,2], B. Rosie E.[4]. [1]Wageningen University, Laboratory of Phytopathology,, [2]Centre for Biosystems Genomics, Wageningen, The Netherlands, [3]Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia, [4]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand

16:50 **CS11-4. Protecting forest crops from disease: can comparative genomics provide management solutions?** R. E. Bradshaw[1], Y. Guo[1], S. Kabir[1], P. Chettri[1], M. P. Cox[1], B. Okmen[2], J. Collemare[2], P. J. G. M. de Wit[2]. [1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand, [2]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

17:10 **CS11-5. Transgenic potato plants expressing WRKY8 transcription factor show resistance to potato blight pathogens.** M. Yoshioka[1], N. Ishihama[2], Y. Kanehara[1], H. Adachi[1], Y. Takano[3], H. Yoshioka[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [2]RIKEN Plant Science Center, Yokohama, Japan, [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

17:30 **CS11-6. Resistance genes within the same TIR-NBS-LRR locus from a wild North American grapevine species confer resistance to powdery mildew and downy mildew in cultivated grapevine.** A. Feechan[1], C. Anderson[1], L. Torregrosa[2], A. Jermakow[1], P. Mestre[3], S. Wiedemann-Merdinoglu[3], D. Merdinoglu[3], A. Walker[1], L. Cadle-Davidson[4], B. Reisch[5], S. Aubourg[6], N. Bentahar[6], B. Shrestha[2], A. Bouquet[2], A.-F. Adam-Blondon[6], M. R. Thomas[1], I. B. Dry[1]. [1]CSIRO Plant Industry, Adelaide, Australia, [2]UMR DIAPC - Campus SupAgro-INRA, place Viala, 34060, Montpellier, France, [3]INRA, Sante Vigne & Qualite Vin UMR1131, F-68000 Colmar, France, [4]USDA-ARS Grape Genetics Research Unit, Geneva, NY, USA, [5]Department of Horticulture, Cornell University, Geneva, NY, USA., [6]INRA-URGV 2, rue Gaston Cremieux CP 5708 F-91057 Evry, France.

Concurrent 12 - Evolution of susceptibility and resistance

15:50 - 17:50; Room D

Co-Chairs: Walter Gassmann, University of Missouri, USA
Gary Stacey, University of Missouri, USA

15:50 **CS12-1. Plant recognition of chitin and lipo-chitin signaling molecules.** G. Stacey[1]. [1]Divisions of Biochemistry and Plant Science, University of Missouri, Columbia, MO, USA

16:10 **CS12-2. Yin and yang of effector-triggered immunity: the negative regulator SRFR1 interacts with the positive regulator EDS1 and with resistance proteins.** S. Bhattacharjee[1], M. Halane[1], S. H. Kim[1,2], W. Gassmann[1]. [1]Division of Plant Sciences, University of Missouri, Columbia, USA, [2]Department of Biology, Indiana University, Bloomington, USA

16:30 **CS12-3. EDS1 connects pathogen effector recognition to cell compartment-specific immune responses.** K. E. Heidrich[1], L. Wirthmueller[2], C. Tasset[3], C. Pouzet[4], L. Deslandes[3], J. E. Parker[1]. [1]Max Planck Institute for Plant Breeding Research, Department of Plant Microbe Interactions, [2]John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK, [3]CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR 2594, F-31326 Castanet-Tolosan, France, [4]Federation de Recherche 3450, Plateforme Imagerie TRI, Pole de Biotechnologie Vegetale, F-31326 Castanet-Tolosan, France

16:50 **CS12-4. *Arabidopsis Non-race specific Resistance-1 Disease (NDR1)* is required for robust activation of drought tolerance and PAMP triggered immunity via an abscisic acid dependent pathway.** P. Ferreira Santos[1], C. Knepper[1], L. Yan[1], E. A. Savory[1], B. Day[1]. [1]Dept of Plant Pathology, Michigan State University, East Lansing, MI, USA

17:10 **CS12-5. Timing of innate immunity by the circadian clock in Arabidopsis.** C. Zhang[1], Q. Xie[2], R. Anderson[3], G. Ng[1], N. Seitz[1], C. R. McClung[2], J. M. McDowell[3], D. Kong[4], J. Kwak[4], H. Lu[1]. [1]Department of Biological Science, University of Maryland Baltimore County, Baltimore, MD, USA, [2]Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755, [3]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061-0323, USA., [4]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA.

17:30 **CS12-6. Repeated evolution of genetic incompatibilities involving a single NB-LRR gene cluster: lessons from hybrid necrosis studies in *Arabidopsis thaliana*.** E. Chae[1], K. Bomblies[1], S.-T. Kim[1], D. Karelina[1], S. Ossowski[1], B. Rowan[1], M. Demar[1], C. Lanz[1], D. Weigel[1]. [1]Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany

Wednesday, August 1

8:00 - 15:30	Registration		Reception, Main Entrance
8:30 - 17:30	Poster viewing		Event Hall
8:30 - 10:00	**Plenary 5**	Plant signaling II	Main Hall
10:00 - 10:20	Coffee Break		
10:20 - 12:20	**Plenary 6**	Plant-microbe interactions II	Main Hall
12:20 - 13:30	Lunch Break		Sakura and Swan
	Exhibits Open and Poster Viewing		Event Hall
13:30 - 15:30	**Concurrent 13**	Plant response	Main Hall
	Concurrent 14	Pathogenic bacteria / phytoplasma	Room A
	Concurrent 15	Systems biology	Room D
15:40 - 20:00	**Excursion**, *pre-registration and ticket required*		
	Pick-up Location and Time: In front of the main entrance of ICC Kyoto, 16:00		

SESSIONS – Wednesday Morning

Plenary 5 - Plant signaling II
8:30 - 10:00 ; Main Hall
Chair: Peter N. Dodds, CSIRO Plant Industry, Australia

8:30 **PL5-1. Recognition of rust effectors in plant innate immunity.** P. Dodds[1], J. Ellis[1], M. Bernoux[1], M. Ravensdale[1], B. Kobe[2], S. Williams[2], T. Ve[2], A. Hardham[3], D. Jones[3], A.-M. Catanzariti[3], M. Rafiqi[3], M. Koeck[1], W. Wu[3]. [1]CSIRO Plant Industry, [2]University of Queensland, School of Chemistry and Molecular Biosciences, [3]Australian National University, Research School of Biology

9:00 **PL5-2. Signaling networks in plant innate immunity.** J. Sheen[1], G. Tena[1], M. Boudsocq[2], H. Lee[1], Y. Xiong[1], M. McCormack[1], Y. Niu[1], J. Bush[1], L. Li[1], L. Shan[3], P. He[4]. [1]The Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA, [2]Unite de Recherche en Genomique Vegetale, INRA-CNRS-UEVE, Cedex, France, [3]Department of Plant Pathology and Microbiology, and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, USA, [4]Department of Biochemistry and Biophysics, and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, USA

9:30 **PL5-3. Molecular basis of ATR1 effector recognition and activation of the RPP1 NLR innate immune receptor complex.** K. Krasileva[1], A. Steinbrenner[1], S. Goritschnig[1], K. Schreiber[1], B. Staskawicz[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720 USA

Plenary 6 - Plant-microbe interactions II
10:20 - 12:20; Main Hall
Chair: Jian-Min Zhou, Chinese Academy of Sciences, China

10:20 **PL6-1. Biochemical functions of bacterial effectors and plant immunity.** J.-M. Zhou[1], C. He[2]. [1]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, [2]Hainan University, Haikou, China

10:50 **PL6-2. Targeting transcription factors: mechanism of effector repression.** M. B. Mudgett[1], J.-G. Kim[1], W. F. J. Stork[1]. [1]Department of Biology, Stanford University, Stanford, CA, USA

11:20 **PL6-3. Signal transduction in plant root symbiosis.** M. Parniske[1]. [1]Faculty of Biology, Genetics, University of Munich (LMU), Germany

11:50 **PL6-4. Establishing beneficial interactions with the symbiosis signalling pathway.** G. Oldroyd[1]. [1]John Innes Centre

SESSIONS – Wednesday Afternoon

Concurrent 13 - Plant response
13:30 - 15:30; Main Hall
Co-Chairs: Gary J. Loake, University of Edinburgh, UK
Hirofumi Yoshioka, Nagoya University, Nagoya, Japan

13:30 **CS13-1. Molecular aspects of defense priming.** U. Conrath[1]. [1]Department of Plant Physiology, RWTH Aachen University, Aachen, Germany

13:50 **CS13-2. Effector modulation of the Arabidopsis actin cytoskeleton by *Pseudomonas syringae*.** B. Day[1], M. Shimono[1], K. Porter[1], A. Creason[2], J. Chang[2]. [1]Michigan State University, [2]Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR

14:10 **CS13-3. Dynamics and biological significance of RNA-directed DNA methylation in plant immunity.** A. Yu[1], G. Lepere[1], F. Jay[2], L. Bapaume[1], J. Wang[1], Y. Wang[3], A.-L. Abraham[1], O. Voinnet[2], L. Navarro[1]. [1]Institut de Biologie de L Ecole Normale Superieure, [2]Swiss Federal Institute of Technology Zurich Department of Biology Zurich Switzerland., [3]Department of Plant Science Center for Life and Food Sciences Weihenstephan Technical University Munich

14:30 **CS13-4. Lack of susceptibility factors: a novel breeding strategy for non-host like resistance?** Y. Bai[1], R. Huibers[1], A. E. H. M. Loonen[1], D. Gao[1], G. van den Ackerveken[2], R. G. F. Visser[1]. [1]Wageningen University, [2]Utrecht University, the Netherlands

14:50 **CS13-5. Molecular mechanisms for generation of NO and ROS in plant immunity.** H. Yoshioka[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

15:10 **CS13-6. Plant immu*NO*logy: Cracking the redox code.** G. J. Loake[1]. [1]IMPS, University of Edinburgh, UK

Concurrent 14 - Pathogenic bacteria / phytoplasma
13:30 - 15:30; Room A
Co-Chairs: Adam Bogdanove, Cornell University, USA, Iowa State University, USA
Saskia A. Hogenhout, The John Innes Centre, UK

13:30 **CS14-1. Harnessing TAL effector-DNA targeting to understand and prevent plant diseases caused by *Xanthomonas*.** A. J. Bogdanove[1,2], R. A. Cernadas[1,2], E. L. Doyle[2], A. W. Hummel[2], C. L. Schmidt[2], L. Wang[1,2]. [1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, [2]Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA USA

13:50 **CS14-2. Phytoplasma effectors modulate plant development and plant-insect interactions.** S. A. Hogenhout[1], A. Sugio[1], A. M. MacLean[1], H. N. Kingdom[1], V. M. Grieve[1]. [1]Department of Cell and Developmental Biology, The John Innes Centre, Norwich, UK

14:10 **CS14-3. The trimeric autotransporter adhesin XadA is localized in outer membrane vesicles and mediates attachment to surfaces and suppress cell-cell aggregation in *Xylella fastidiosa*.** M. Ionescu[1], A. M. da Silva[1,2], C. Baccari[1], A. A. de Souza[3], N. Killiny[4], R. P. P. Almeida[4], S. E. Lindow[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley, USA, [2]Department of Biochemistry, Universidade de Sao Paulo, Sao Paulo, Brazil, [3]IAC-Centro APTA Citros Sylvio Moreira, Cordeiropolis, Brazil, [4]Department of Environmental Science, Policy, and Management, University of California, Berkeley, USA

14:30 **CS14-4. Towards a life history model of *Pseudomonas syringae* pv. *tomato* that integrates adaptations to habitats beyond the plant apoplast.** B. A. Vinatzer[1], R. Cai[1], C. R. Clarke[1], C. L. Monteil[2], D. J. Studholme[3], C. E. Morris[2]. [1]PPWS Department, Virginia Tech, Blacksburg, Virginia, USA, [2]INRA Centre de Recherche en PACA, Plant Pathology Research Unit, Montfavet, France, [3]University of Exeter, Exeter, Devon, UK

14:50 **CS14-5. Systematic dissection of the *Agrobacterium* type VI secretion system reveals machinery and secreted components for subcomplex formation.** J.-S. Lin[1], L.-S. Ma[1,2,3], E.-M. Lai[1,2,3]. [1]Institute of Plant and Microbial Biology , Academia Sinica, Taipei, Taiwan, [2]Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, National Chung-Hsing University and Academia Sinica, Taipei, Taiwan., [3]Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan.

15:10 **CS14-6. Isolation of *Burkholderia glumae* resistance genes from rice using whole genome association mapping.** S. Sharma[1], S. Sharma[1], H. Saitoh[1], H. Takagi[1], A. Abe[1], M. Tamiru Oli[1],

R. Fekih Ep Laribi[1], R. Terauchi[1]. [1]Rice Genetics and Genomics, Iwate Biotechnology Institute, Kitakami, Iwate, Japan

Concurrent 15 - Systems biology
13:30 - 15:30; Room D
Co-Chairs: Fumiaki Katagiri, University of Minnesota, USA
Roger Wise, U.S. Department of Agriculture-Agricultural Research Service, USA

13:30 **CS15-1. Regulation of innate immunity in barley-powdery mildew interactions.** R. Wise[1], P. Surana[2], G. Fuerst[1], D. Nettleton[3], L. Wang[4], T. Brutnell[4]. [1]Crop and Insect Genetics, Genomics, and Informatics Research Unit, U.S. Department of Agriculture-Agricultural Research Service, [2]Bioinformatics and Computational Biology Graduate Program, Iowa State University, Ames, Iowa 50011, [3]Department of Statistics, Iowa State University, Ames, Iowa 50011, [4]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853

13:50 **CS15-2. Properties and structure of the plant immune signaling network.** F. Katagiri[1], K. Tsuda[1,2], Y. Kim[3], R. Hillmer[1], D. Igarashi[1,4], C. Myers[3]. [1]Department of Plant Biology, Microbial and Plant Genomics Institute, University of Minnesota, MN, USA, [2]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [3]Department of Computer Science and Engineering, Microbial and Plant Genomics Institute, University of Minnesota, MN, USA, [4]Institute for Innovation, Ajinomoto Co., Inc., Kawasaki, Japan

14:10 **CS15-3. Rapid Nod factor-induced changes in the phosphoproteome and the transcriptome of *Medicago truncatula*.** M. Venkateshwaran[1], C. M. Rose[2,4], J. D. Volkening[3], P. A. Grimsrud[3], J. Maeda[1], D. J. Bailey[2,4], K. Park[4,5], M. Howes-Podoll[1], M. S. Westphall[2,4], J. J. Coon[2,4,6], M. R. Sussman[3,4], J.-M. Ane[1]. [1]Department of Agronomy, University of Wisconsin, Madison, WI-53706, USA, [2]Department of Chemistry, University of Wisconsin, Madison, WI-53706, USA, [3]Department of Biochemistry, University of Wisconsin, Madison, WI-53706, USA, [4]Genome Center of Wisconsin, University of Wisconsin, Madison, WI-53706, USA, [5]Department of Computer Sciences, University of Wisconsin, Madison, WI-53706, USA, [6]Department of Biomolecular Chemistry, University of Wisconsin, Madison, WI-53706, USA

14:30 **CS15-4. Ceramide accumulation plays a key role in Arabidopsis programmed cell death.** F. Bi[1], Z. Liu[1], H. Liang[2], X. Xi[1], F. Ce[1], J. T. Greenberg[2], N. Yao[1]. [1]School of Life Sciences, Sun Yat-sen University, [2]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois

14:50 **CS15-5. Two novel transcription factors regulating MAMP-elicited phenylpropanoid metabolism in Arabidopsis.** W. R. Chezem[1]. [1]Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, United States

15:10 **CS15-6. Signaling in soybean defense responses.** S. A. Whitham[1], J.-Z. Liu[1], C. Zhang[1], M. A. Graham[2], J. H. Hill[1]. [1]Department of Plant Pathology & Microbiology, Iowa State University, Ames, Iowa, USA, [2]Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, Iowa, USA

Thursday, August 2

8:00 - 12:00	Registration		Reception, Main Entrance
8:30 - 12:20	Poster viewing		Event Hall
8:30 - 10:00	**Plenary 7**	Plant immunity II	Main Hall
10:00 - 10:20	Coffee Break		
10:20 - 12:20	**Concurrent 16**	Recognition and signaling II	Main Hall
	Concurrent 17	Symbiosis II	Room A
	Concurrent 18	Endophytes and parasitic plants	Room D
12:20 - 13:30	Lunch Break		Sakura and Swan
	Poster take-down and Exhibitor move-out		Event Hall
13:30 - 15:30	**Concurrent 19**	Biotechnology	Main Hall
	Concurrent 20	Genomics and evolution of virulence in pathogenic fungi and oomycetes	Room A
	Concurrent 21	Structural biology	Room D
15:30 - 15:50	Coffee Break		
15:50 - 17:50	**Plenary 8**	Plant-microbe interactions III	Main Hall
18:00 - 18:30	**Closing Ceremony**		Main Hall
19:00 - 21:00	**Congress Dinner**, *pre-registration and ticket required*		Prince Hall (Grand Prince Hotel Kyoto)

SESSIONS – Thursday Morning

Plenary 7 - Plant immunity II

8:30 - 10:00; Main Hall

Chair: John Rathjen, The Australian National University, Australia

8:30 **PL7-1. Pathogen effector proteins and pathogenicity on plants.** J. Rathjen[1]. [1]Research School of Biology, The Australian National University, Canberra, Australia

9:00 **PL7-2. Effectors in smut fungi and how they affect virulence.** R. Kahmann[1]. [1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

9:30 **PL7-3. Bacterial manipulation of jasmonate receptor and immunity in plants.** S. Y. He[1]. [1]Michigan State University

Concurrent 16 - Recognition and signaling II

10:20 - 12:20; Main Hall

Co-Chairs: Gitta Coaker, University of California Davis, USA
Peter Moffett, University of Sherbrooke, Canada

10:20 **CS16-1. Involvement of a novel class of NB-LRR proteins in disease resistance.** P. Moffett[1]. [1]Department of Biology, University of Sherbrooke, Sherbrooke, Quebec, Canada

10:40 **CS16-2. Proteomic and genetic analyses of plant immune complexes.** G. Coaker[1]. [1]University of California Davis

11:00 **CS16-3. Danger sensing and signaling via an endogenous elicitor/receptor system in *Arabidopsis*.** K. Yamada[1], A. Ross[1], N. Tintor[1], M. Yamashita-Yamada[1], Y. Saijo[1]. [1]Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany

11:20 **CS16-4. Lectin-mediated resistance as a novel and universal innate immunity toward plant viruses.** Y. Yamaji[1], K. Maejima[1], K. Komatsu[1], T. Shiraishi[1], Y. Okano[1], M. Himeno[1], K. Sugawara[1], Y. Neriya[1], N. Minato[1], C. Miura[1], M. Hashimoto[1], S. Namba[1]. [1]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

11:40 **CS16-5. Interplays between positive and negative transcriptional regulators mould NB-LRR protein triggered immunity.** C. Chang[1], D. Yu[1], J. Jiao[1], S. Jing[1], P. Schulze-Lefert[2], Q.-H. Shen[1]. [1]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, [2]Dept of Plant Microbe Interactions, Max-Planck Institut Pflanzenzuchtungsforschung, Cologne, Germany

12:00 **CS16-6. mRNA decay in kinase-mediated responses to pathogens.** M. Roux[1], K. Palma[1], M. Rasmussen[1], L. Arribas[1], J. Mundy[1], M. Petersen[1]. [1]Department of Plant Molecular Biology,

University of Copenhagen, Copenhagen, Denmark

Concurrent 17 - Symbiosis II
10:20 - 12:20; Room A
Co-Chairs: Makoto Hayashi, National Institute of Agrobiological Sciences, Japan
Uta Paszkowski, University of Lausanne, Switzerland

10:20 **CS17-1. Transcriptional regulation for nodulation in legumes.** M. Hayashi[1], T. Soyano[1]. [1]National Institute of Agrobiological Sciences

10:40 **CS17-2. Identification of a common regulator involved both in nodulation and shoot apical meristem maintenance in *Lotus japonicus*.** T. Suzaki[1,2], C. S. Kim[3], N. Takeda[1,2], K. Szczyglowski[3], M. Kawaguchi[1,2]. [1]National Institute for Basic Biology, [2]School of Life Science, Graduate University for Advanced Studies (SOKENDAI), [3]Southern Crop Protection and Food Research Centre, Canada

11:00 **CS17-3. Regulation of *Medicago truncatula* HMGR1 by symbiotic receptor-like kinases and its role in early symbiotic signaling.** J.-M. Ane[1], D. Jayaraman[1], K. L. Forshey[1], M. Venkateshwaran[1], B. K. Riely[2], E. Larrainzar[2], M. Howes-Podoll[1], D. R. Cook[2]. [1]Department of Agronomy, University of Wisconsin - Madison, Madison, Wisconsin 53706, USA, [2]Department of Plant Pathology, University of California - Davis, Davis, California 95616, USA

11:20 **CS17-4. New roles for strigolactones in legume symbioses.** E. Foo[1], C. Hugill[1], L. Quittenden[1], J. B. Reid[1], K. Yoneyama[2]. [1]School of Plant Science, University of Tasmania, Tasmania, Australia, [2]Weed Science Center, Utsunomiya University, Japan

11:40 **CS17-5. Intracellular accommodation of microbes by plants: Novel systems to study commonalities and differences between symbionts and pathogens.** S. Schornack[1], E. Wang[2], A. Breakspear[2], J. Murray[2], G. Oldroyd[2], S. Kamoun[1]. [1]The Sainsbury Laboratory, Norwich, UK, [2]John Innes Centre, Norwich, UK

12:00 **CS17-6. Essential factors for arbuscular mycorrhizal symbiosis: lessons from maize and rice.** M. Nadal[1], R. Sawers[1], C. Gutjahr[1], S.-Y. Yang[1], K. An[2], G. An[2], K. Ahern[3], T. Brutnell[3], U. Paszkowski[1]. [1]Department of Plant Molecualr Biology, University of Lausanne, Lausanne, Switzerland, [2]Crop Biotech Institute and Department of Plant Molecular Systems Biotechnology, Kyung Hee University, Yongin, Korea, [3]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York, USA

Concurrent 18 - Endophytes and parasitic plants
10:20 - 12:20; Room D
Co-Chairs: Kiwamu Minamisawa, Tohoku University, Japan
Ken Shirasu, RIKEN, Japan

10:20 **CS18-1. What does community analysis of plant-associated microbes tell us?** K. Minamisawa[1], S. Ikeda[2], T. Okubo[1], M. Anda[1], K. Sasaki[1], Z. Bao[1], T. Sato[1], H. Imaizumi-Anraku[3]. [1]Graduate School of Life Sciences, Tohoku University, [2]Memuro Research Station, National Agricultural Research Center for Hokkaido Region, [3]Department of Plant Sciences, National Institute of Agrobiological Sciences

10:40 **CS18-2. Factors affecting endophytic colonization of rice.** B. Reinhold-Hurek[1], T. Shidore[1], T. Dinse[1], H. Klingenberg[1]. [1]Dept. of Microbe-Plant Interactions, Uni versity of Bremen, Bremen, Germany

11:00 **CS18-3. Effects of colonization of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in *Arabidopsis*.** M. Yasuda[1], J. Hirayama[1,2,3], K. Minamisawa[3], S. Shinozaki[1,3], H. Nakashita[1,4]. [1]Plant-Endophyte Interactions Laboratory, RIKEN Innovation Center, Saitama, Japan, [2]Plantech Research Institute, Mayekawa MFG. Co., Ltd., Shizuoka, Japan, [3]Laboratory of Environmental Plant Microbiology, Graduate School of Life Sciences, Tohoku University, Sendai, Japan, [4]Department of Applied Biology and Chemistry, Tokyo University of Agriculture, Tokyo, Japan

11:20 **CS18-4. Genomic and transcriptomic analyses of the parasitic plants.** K. Shirasu[1], S. Yoshida[1], J. Ishida[1], R. Manabe[2]. [1]RIKEN, Plant Science Center, Yokohama, Japan, [2]RIKEN Omics Science Center, Yokohama, Japan

11:40 **CS18-5. Reduced exudation of 5-deoxystrigol confers resistance to *Striga* in maize cultivars.** K. Yoneyama[1], R. Arakawa[2], X. Xie[1], T. Kisugi[1], T. Nomura[1], T. Ezawa[2], K. Yoneyama[1]. [1]Weed Science Center, Utsunomiya University, Utsunomiya, Japan, [2]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

12:00 **CS18-6. Nitrogen fluxes in the *Phelipanche ramosa* / *Brassica napus* interaction.** Z. Gaudin[1], J.-B. Pouvreau[1], R. J. Robins[2], P. Delavault[1], P. Simier[1]. [1]Plant Biology and Pathology Laboratory, University of Nantes, Nantes, France, [2]Chemistry and Interdisciplinarity Laboratory : Synthesis, Analysis, Modelisation. University of Nantes, Nantes, France

SESSIONS – Thursday Afternoon

Concurrent 19 - Biotechnology (Sponsored by the Two Blades Foundation)
13:30 - 15:30; Main Hall
Co-Chairs: Eric Ward, Two Blades Foundation, USA, The Sainsbury Laboratory, UK
Zhongmin Wei, Plant Health Care Inc., USA

13:30 **CS19-1. Harpin, elicitor of hypersensitive response for new era agricultural application-opportunities and challenges.** Z. Wei[1]. [1]Plant Health Care Inc.

13:50 **CS19-2. Toward durable disease resistance to wheat rusts.** B. Wulff[2], M. Moscou[2], N. Champouret[2], D. Horvath[2], J. Kaufman[3], B. Steffenson[3], E. Ward[1,2]. [1]Two Blades Foundation, [2]The Sainsbury Laboratory, [3]University of Minnesota

14:10 **CS19-3. Addition of TAL effector binding sites to a pathogen strain-specific rice bacterial blightresistance gene makes it effective against additional strains and against bacterial leaf streak.** A. W. Hummel[1], E. L. Doyle[1], A. J. Bogdanove[1]. [1]Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa

14:30 **CS19-4. Effector-driven disease resistance breeding in potato.** V. G. A. A. Vleeshouwers[1]. [1]Wageningen UR Plant Breeding, Wageningen University & Research Centre, Wageningen, The Netherlands

14:50 **CS19-5. Application of MutMap to identify rice genes involved in blast resistance.** A. Abe[1], S. Kosugi[1], K. Yoshida[1], S. Natsume[1], H. Takagi[1,2], H. Kanzaki[1], H. Matsumura[1,3], K. Yoshida[1], C. Mitsuoka[1], M. Tamiru[1], H. Innan[4], L. Cano[5], S. Kamoun[5], R. Terauchi[1]. [1]Iwate Biotechnology Research Center, [2]United Graduate School of Agricultural Science, Iwate University, [3]Gene Research Center, Shinshu University, [4]The Graduate University for Advanced Studies, [5]The Sainsbury Laboratory

15:10 **CS19-6. A polygalacturonase inhibitor confers to transgenic tobacco resistance against fungi and oomycetes.** F. Cervone[1], O. Borras-Hidalgo[2], C. Caprari[3], M. Benedetti[1], G. De Lorenzo[1]. [1]Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Italy, [2]Center of Biotechnology and Genetic Engineering, La Habana, Cuba, [3]Department STAT, Università del Molise, Pesche (IS), Italy

Concurrent 20 - Genomics and evolution of virulence in pathogenic fungi and oomycetes
13:30 - 15:30; Room A
Co-Chairs: Christina A. Cuomo, Broad Institute of MIT and Harvard, USA
Brett M. Tyler, Oregon State University, USA, Virginia Tech, USA

13:30 **CS20-1. Life-style transitions in hemibiotrophic *Colletotrichum* fungi uncovered by comparative genome and transcriptome analyses.** R. O'Connell[1], M. Thon[2], S. Hacquard[1], J. Kleemann[1], S. Amyotte[3], M.-H. Lebrun[4], E. Ver Loren van Themaat[1], L.-J. Ma[5], L. Vaillancourt[3]. [1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Salamanca, Spain, [3]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, USA, [4]INRA BIOGER, Thiverval-Grignon, France, [5]The College of Natural Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

13:50 **CS20-2. Global reprogramming of DNA methylation during pathogenic development in the rice blast fungus.** J. Jeon[1], J. Choi[1], G.-W. Lee[1], S.-Y. Park[1], A. Huh[1], R. Dean[2], Y.-H. Lee[1]. [1]Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea, [2]Department

of Plant Pathology, North Carolina State University, Raleigh, NC, USA

14:10 **CS20-3. Genomic evolution and specialization of wheat rust fungi.** C. A. Cuomo[1], S. Sakthikumar[1], J. Goldberg[1], S. Young[1], G. Bakkeren[2], X. Chen[3,4], S. Hulbert[4], L. Szabo[5], J. Fellers[6]. [1]Broad Institute of MIT and Harvard, Cambridge, MA U.S.A., [2]Agriculture & Agri-Food Canada, Summerland, BC, Canada, [3]USDA-ARS, Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA, U.S.A, [4]Washington State University, Pullman, WA, U.S.A, [5]USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St Paul, MN, U.S.A, [6]USDA-ARS, Kansas State University, Manhattan, KS, U.S.A

14:30 **CS20-4. Evolution of cell entry function in oomycete and fungal effectors.** B. M. Tyler[1,2], S. D. Kale[2], V. Antignani[2], J. Vega-Arreguin[2], Z. Shi[3], R. Anderson[2], Q. Wang[1], B. Gu[2,4], D. G. S. Capelluto[2], D. Dou[2,8], A. Rumore[2], J. Plett[5], R. Aggarwal[6], T. Rouxel[7], F. Martin[5], J. J. Stuart[6], J. McDowell[2], C. B. Lawrence[2], W. Shan[4], M. Guiltinan[3]. [1]Oregon State University, [2]Virginia Tech, Blacksburg, Virginia, USA, [3]Pennsylvania State University, State College, Pennsylvania, USA, [4]NW Agricultural and Forestry University, Yangling, China, [5]Centre INRA de Nancy, Champenoux, France, [6]Purdue University, West Lafayette, Indiana, USA, [7]INRA-Bioger, Campus AgroParisTech, Thiverval-Grignon, France, [8]Nanjing Agricultural University, Nanjing, China

14:50 **CS20-5. Mining the *Rhynchosporium commune* genome and transcriptome for pathogenicity determinants.** A. Avrova[1]. [1]Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK

15:10 **CS20-6. Identifying effector proteins in two fungal pathogens of *Brassica napus*.** R. G. T. Lowe[1], A. P. Van de Wouw[1], A. Cassin[1], B. Howlett[1]. [1]The School of Botany, University of Melbourne, Melbourne, Australia

Concurrent 21 - Structural biology

13:30 - 15:30; Room D

Co-Chairs: Jijie Chai, Tsinghua University, China
Bostjan Kobe, University of Queensland, Australia

13:30 **CS21-1. Chitin-induced dimerization activates a plant immune receptor.** T. Liu[1], Z. Liu[3], C. Song[2], Y. Hu[5], C. Jin[5], J. Chang[2], J.-M. Zhou[5], J. Chai[1]. [1]Tsinghua University, [2]Zhengzhou University, [3]Nanjing University, [4]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, [5]Peking University

13:50 **CS21-2. Structural basis of dual R protein signalling in Arabidopsis.** S. J. Williams[1], L. Wan[1], T. Ve[1], M. Bernoux[2], J. G. Ellis[2], P. N. Dodds[2], B. Kobe[1,3]. [1]School of Chemistry and Molecular Biosciences and Centre for Infectious Disease Research, University of Queensland, Brisbane, Queensland 4072, Australia, [2]CSIRO Plant Industry, Canberra, Australian Capital Territory 2601, Australia, [3]Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland 4072, Australia

14:10 **CS21-3. Crystal structure and interaction with host factors of the superfamily 1 helicase from *Tomato mosaic virus*.** E. Katoh[1], M. Nishikiori[1], S. Sugiyama[2], H. Xiang[1], M. Niiyama[2], K. Ishibashi[1], T. Inoue[2], H. Matsumura[2], M. Ishikawa[1]. [1]National Institute of Agrobiological Sciences, [2]Osaka University

14:30 **CS21-4. Structure-led studies of oomycete RXLR effectors: a conserved protein fold and new host targets.** S. R. F. King[1], L. S. Boutemy[1], R. K. Hughes[1], J. Win[2], S. Kamoun[2], M. J. Banfield[1]. [1]Dept. of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK, [2]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

14:50 **CS21-5. Structural analysis of the flax-rust effector AvrM.** T. Ve[1], S. Williams[1], J. G. Ellis[2], P. N. Dodds[2], P. A. Anderson[3], B. Kobe[1,4]. [1]School of Chemistry and Molecular Biosciences, and Centre for Infectious Disease Research, University of Queensland, Australia, [2]CSIRO Plant Industry, Canberra, Australian Capital Territory 2601, Australia, [3]The School of Biological Sciences, Flinders University, Adelaide, Australia, [4]Institute for Molecular Bioscience (Division of Chemistry and Structural Biology), University of Queensland, Australia

15:10 **CS21-6. Protease-inhibitor arms-races in the tomato apoplast.** A. C. Hoerger[1], M. Ilyas[1], S. Kumari[1], F. Kaschani[1], M. Shabab[1], M. Smoker[2], M. H. A. J. Joosten[3], L. E. Rose[4], S. Kamoun[2], R. A. L. van der Hoorn[1]. [1]Plant Chemetics lab, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Sainsbury laboratory, John Innes Centre, Norwich, United Kingdom, [3]Phytopathology laboratory, Wageningen University, Wageningen, The Netherlands, [4]Institute for Population Genetics, Heinrich Heine University, Dusseldorf, Germany

Plenary 8 - Plant-microbe interactions III
15:50 - 17:50; Main Hall
Chair: Thomas Lahaye, Ludwig-Maximilians-University Munich, Germany

15:50 **PL8-1. RNA-seq identifies a novel *Xanthomonas* specific plant resistance gene in pepper.** T. Lahaye[1]. [1]Ludwig-Maximilians-University Munich

16:20 **PL8-2. The role of LysM type receptors in Nod factor perception.** J. Stougaard[1]. [1]Department of Molecular Biology and Genetics, Aarhus University, Denmark

16:50 **PL8-3. Virus and plant endogenous siRNAs in antiviral responses and pathogen discovery.** S.-W. Ding[1]. [1]Department of Plant Pathology & Microbiology & Institute for Integrative Genome Biology, University of California, Riverside, California, USA

17:20 **PL8-4. Plant volatiles drive ecological interaction networks.** J. Takabayashi[1]. [1]Center for Ecological Research, Kyoto University, Shiga, Japan

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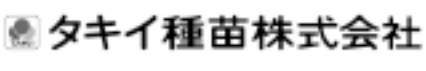

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IS-MPMI XV CONGRESS POSTERS *Pages 38-85*

Poster Schedule and Poster Titles by Category
Taking photographs of material projected during presentations or displayed is prohibited.

Poster Hours

Sunday, July 29	14:00 - 17:30	Poster set-up
Monday, July 30	8:30 - 20:30	Poster viewing
	18:00 - 20:00	Odd-numbered poster authors present
Tuesday, July 31	8:30 - 20:30	Poster viewing
	18:00 - 20:00	Even-numbered poster authors present
Wednesday, August 1	8:30 - 17:30	Poster viewing
Thursday, August 2	8:30 - 12:20	Poster viewing
	12:20 - 13:30	Poster take-down

Presentation Categories

Poster Titles and Authors

Affiliation is indicated as in the abstract.

Recognition and signaling I / II

PS01-001. **Defence signaling triggered by flg22 and Harpin diverge at stilbenic biosynthesis in *Vitis* cells.** X. Chang[1], P. Nick[1]. [1]Botanical Institute, Karlsruhe Institute of Technology, Karlsruhe, Germany

PS01-002. **Is Peptidoglycan recognized in plants via a LysM-protein receptor complex?** Y. Desaki[1], R. Willmann[1], H. M. Grabherr[1], D. Kolb[1], A. A. Gust[1], T. Nuernberger[1]. [1]Center for Plant Molecular Biology, Department of Plant Biochemistry, University of Tuebingen, Tuebingen, Germany

PS01-003. **The role of antisense transcription in the quorum sensing regulation in *Pectobacterium atrosepticum* SCRI1043.** Y. V. Gogolev[1], V. Y. Gorshkov[1], L. V. Shlykova[1], N. E. Gogoleva[1]. [1]Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia

PS01-004. **Revealing mechanisms underlying conserved MLA-mediated immunity in monocots and dicots by interfamily gene transfer.** T. Maekawa[1], F. Jacob[1], S. Vernaldi[1], P. Schulze-Lefert[1]. [1]Max Planck Institute for Plant Breeding Research, Cologne, Germany

PS01-005. **Analysis of the Defensome complex in rice innate immunity.** S. Hamada[1], M. Fujiwara[1], K. Shimamoto[1]. [1]Nara Institute of Science and Technology, Ikoma, Nara, Japan

PS01-006. **Disruption of sphingolipid biosynthesis in *Nicotiana benthamiana* activates salicylic acid-dependent responses and compromises resistance to *Alternaria alternata f. sp. lycopersici*.** J. Plasencia[1], M. Rivas-San Vicente[1], G. Larios-Zarate[1]. [1]Dept. Bioquimica, Facultad de Quimica, Universidad Nacional Autonoma de Mexico

PS01-007. **Reduction of sphingolipid 2-hydroxy fatty acids has an impact on defense response through decrease of membrane rafts in rice.** M. Nagano[1], T. Ishikawa[2], M. Kawai-Yamada[2], K. Shimamoto[1]. [1]Graduate school of Biological Science, Nara Institute of Science and Technology, Nara, Japan, [2]Graduate school of Environmental Science and Human Engineering, Saitama University, Saitama, Japan

PS01-008. **A MACPF protein is required for cell death regulation in biosynthesis of antifungal compounds in *Arabidopsis*.** S. Fukunaga[1], M. Sogame[1], M. O. Komori[1], H. Saitoh[2], R. Terauchi[2], T. Okuno[1], Y. Takano[1]. [1]Laboratory of Plant Pathology, Graduate School of Agricultute, Kyoto University, kyoto, Japan, [2]Iwate Biotechnology Research Center, Kitakami, Iwate, Japan

PS01-009. **FMO1 and ALD1 mediate a common NPR1-dependent and SA-independent defence signal.** A. Lenk[1], C. Pedersen[1], H. Thordal-Christensen[1]. [1]Department of Sciences, University of Copenhagen, Frederiksberg, Denmark

PS01-010. **Development of Raichu FRET sensors to monitor the immune responses in *Arabidopsis thaliana*.** M. Higuchi[1], K. Shimamoto[1]. [1]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan

PS01-011. **NbMIP1, a J-domain Protein, is required for both *Tobacco mosaic virus* infection and plant disease resistance.** Y. Du[1], J. Zhao[1], H. Zhang[1], T. Chen[1], Y. Liu[1]. [1]Tsinghua University

PS01-012. **The *Magnaporthe oryzae* effectors AvrCO39 and Avr-Pia are recognized by the rice Nucleotide Binding-Leucine rich repeat (NB-LRR) protein RGA5 through direct interaction.** S. Cesari[1], I. Abidi[1], V. Chalvon[1], J.-B. Morel[1], R. Terauchi[2], T. Kroj[1]. [1]INRA, Laboratory of Biology and Genetics of Plant-Pathogen Interaction, [2]Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan

PS01-013. **Lipid modification of the NB-LRR-type R protein Pit is required for its localization to the plasma membrane and immune responses.** Y. Kawano[1], A. Akamatsu[1], A. Yao[1], Y. Housen[1], K. Shimamoto[1]. [1]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, Nara, Japan

PS01-014. **Plant immune receptors: what are the first steps that trigger defence signalling?** M. Bernoux[1], S. Williams[2], J. G. Ellis[1], B. Kobe[2], P. N. Dodds[1]. [1]CSIRO, Plant Industry, Canberra, Australia, [2]School of Chemistry and Molecular Biociences, University of Queensland, Brisbane, Australia

PS01-015. **The CERK1-RacGEF-OsRac1 pathway is involved in chitin-induced immunity in rice.** A. Akamatsu[1], H. L. Wong[2], J. Okuda[1], K. Nishide[1], K. Imai[1,3], Y. Kawano[1], N. Shibuya[4], T. Kawasaki[1], K. Shimamoto[1]. [1]Nara Institute of Science and Technology, Nara, Japan, [2]Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia, [3]Biological Laboratory, Kansai Medical University, Japan, [4]Department of Life Sciences, Meiji University, Japan, [5]Department of Advanced Bioscience, Kinki University, Japan.

PS01-016. **The Gac-Rsm and SadB signal transduction pathways converge on AlgU to repress flagellar synthesis in the rhizobacterium *Pseudomonas fluorescens* F113.** F. Martinez-Granero[1], A. Navazo[1], E. Barahona[1], M. Redondo-Nieto[1], R. Rivilla[1], M. Martin[1]. [1]Departamento de Biologia, Universidad Autonoma de Madrid

PS01-017. **SUMO-mediated transcriptional reprogramming in plant stress and innate immune responses.** H. A. van den Burg[1], V. Hammoudi[1], M. J. Mazur[1], G. Vlachakis[1]. [1]Molecular Plant Pathology, University of Amsterdam, Amsterdam, The Netherlands

PS01-018. **The *Arabidopsis* endogenous elicitor/receptor Pep/PEPR pathway links different branches of plant immunity.** A. Ross[1], K. Yamada[1], K. Hiruma[1,3], M. Yamashita-Yamada[1], Y. Takano[3], B. Kemmerling[2], T. Nuernberger[2], Y. Saijo[1]. [1]Department of Plant-Microbe Interactions, Max-Planck-Institute for Plant Breeding Research, Cologne 50829, Germany, [2]Department of Plant Biochemistry, Center for Plant Molecular Biology, University of Tuebingen, D-72076 Tuebingen, Germany, [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

PS01-019. **Ethylene and endogenous elicitor/receptor signalling serve at a post-recognition step in MAMP-triggered immunity.** N. Tintor[1], A. Ross[1], K. Kanehara[1], Y. Saijo[1]. [1]Department of Plant-Microbe Interactions, Max-Planck-Institute for Plant Breeding Research, Cologne 50829, Germany

PS01-020. **Identification of PTI signaling components through a suppressor screen using the novel allele *bak1-5*.** J. Monaghan[1], A. Matei[1], H. Rovenich[1], F. Gro Malinovsky[1], O. Shorinola[1], C. Zipfel[1]. [1]The Sainsbury Laboratory, Norwich, UK

PS01-021. **Arabidopsis NIFC1, a component of SCF E3 ligase, implies to act as a negative regulator in plant immunity.** H. H. Sun[1], S. Maekawa[1], Y. Maruyama[1], T. Sato[1], J. Yamaguchi[1]. [1]Graduate School of Life Science, University of Hokkaido , Sapporo,Japan.

PS01-022. **Spatial and temporal cellular dynamics of the Arabidopsis flagellin receptor FLS2 reveal endosomal sorting depending on activation status.** M. Beck[1], J. Zhou[1], D. MacLean[1], S. Robatzek[1]. [1]The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

PS01-023. **Tracking DIR1 movement and investigating the role of DIR1-like during Systemic Acquired Resistance in Arabidopsis.** R. Cameron[1], M. Champigny[1], M. Melas[1], P. Carella[1], D. Wilson[1]. [1]Department of Biology, McMaster University, Hamilton, Ontario, Canada

PS01-024. **Expanding the paradigm of flagellin-triggered immunity: the importance of epitopes beyond flg22 and allelic diversity in both plant receptors and bacterial flagellins.** C. R. Clarke[1], S. Leman[2], K. Scheibel[3], F. Taguchi[5], R. Miki[5], D. Chinchilla[6], G. Felix[7], G. B. Martin[3,4], B. A. Vinatzer[1]. [1]Department of Plant Pathology, Virginia Tech, Blacksburg, VA, [2]Department of Statistics, Virginia Tech, [3]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, [4]Boyce Thompson Institute for Plant Research, [5]Graduate School of Natural Science and Technology, Okayama University, [6]Zurich-Basel Plant Science Center, Botanical Institute, University Basel, [7]Zentrum fur Molekularbiologie der Pflanzen, University Tubingen

PS01-025. **Functional analysis of a Rice AAA-Type ATPase, an attentuator of programmed cell death.** H. L. Wong[1], M. Isshiki[2], P. C. Loh[1], W. K. Toh[1], T. Kawasaki[3], K. Shimamoto[4]. [1]Department of Biological Science, Universiti Tunku Abdul Rahman, Kampar, Malaysia, [2]Kihara Institute for Biological Research, Yokohama City University, Yokohama, Japan, [3]Department of Bioscience, Kinki University, Nara, Japan, [4]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, Ikoma, Japan

PS01-026. **Arabidopsis transcriptional repressor ERF9 participates in resistance against necrotrophic fungi mediated by the DEAR1.** J. Yamaguchi[1], Y. Maruyama[1]. [1]Faculty of Science, Hokkaido University, Sapporo, Japan

PS01-027. **Conventional and unconventional functions of NLR immune receptors in *Arabidopsis*.** V. Bonardi[1], M. Roberts[1], A. Stallmann[1], J. Dangl[1]. [1]University of North Carolina, Chapel Hill, NC, USA

PS01-028. **Short chitin oligomers from arbuscular mycorrhizal fungi trigger NFP-independent Ca^{2+} spiking in *Medicago truncatula* roots.** A. Genre[1], M. Chabaud[2], C. Balzergue[3], V. Puech-Pages[3], S. Rochange[3], G. Becard[3], P. Bonfante[1], D. G. Barker[2]. [1]Departmento of Life Science and Systems Biology, University of Torino, Torino, Italy, [2]Laboratory of Plant Microbe Interactions, UMR CNRS-INRA 259 441, Castanet Tolosan, France, [3]Plant Science Research Laboratory, UMR 5546 UPS CNRS, Castanet Tolosan, France

PS01-029. ***Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection.** R. Willmann[1], H. M. Grabherr[1], D. Kolb[1], A. Molinaro[2], M.-A. Newman[3], A. A. Gust[1], T. Nuernberger[1]. [1]Department of Plant Biochemistry, Center for Plant Molecular Biology, University of Tuebingen, Germany, [2]Dipartimento di Chimica Organica e Biochimica, Universita di Napoli Federico II, Napoli 80126, Italy, [3]Department of Plant Biology and Biotechnology, University of Copenhagen, 1871 Frederiksberg, Denmark

PS01-030. **Toxin-mediated release of DAMPs - A novel trigger of plant innate immunity.** H. Boehm[1], I. Kuefner[1], Z. Kikic[1], L. Toliashvili[1], C. Oecking[1], T. Nuernberger[1]. [1]ZMBP Center For Plant Molecular Biology, Eberhard Karls University Tuebingen, Tuebingen, Germany

PS01-031. **A simple model system for detecting metabolic changes in symbiotic *Nostoc punctiforme*.** D. A. Richter[1], H. Jenke-Kodama[1]. [1]Okinawa Institute of Science and Technology, Okinawa, Japan.

PS01-032. **Characterization of rice chitin elicitor receptor complex.** H. Kaku[1], Y. Sato[1], K. Sato[1], D.

Takamizawa[1], T. Shinya[1], M. Hayafune[1], N. Shibuya[1]. [1]Department of Life Sciences, School of Agriculture, Meiji University, Kawasaki, Japan

PS01-033. **Understanding Cf-9 signal activation through molecular genetic dissection of an autoactive mutant, M205.** K. C. Y. Tee[1], C. L. Anderson[1], D. A. Jones[1]. [1]Division of Plant Sciences, Research School of Biology, The Australian National University, ACT, Australia

PS01-034. **The N-terminal MAPK-docking site in tomato MAPK kinase SlMKK2 is required for interaction with a downstream MAPK to trigger programmed cell death associated with plant immunity.** C.-S. Oh[1], M.-S. Choi[1], G. B. Martin[2,3,4]. [1]Department of Horticultural Biotechnology, Kyung Hee University, South Korea, [2]Boyce Thompson Institute for Plant Research, Ithaca, NY, USA, [3]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA, [4]Genomics and Biotechnology Section, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Saudi Arabia

PS01-035. **Identification and molecular characterization of novel PAMPs from the gram-negative bacterium *Ralstonia solanacearum* in *Arabidopsis thaliana*.** E. Melzer[1]. [1]Center for Plant Molecular Biology

PS01-036. **Cysteine-rich receptor like kinase family members are differentially activated by powdery mildew infection in susceptible and mlo-resistant barley.** M. Lyngkjaer[1], D. B. Collinge[1], C. G. Rayapuram[1]. [1]Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Copenhagen, Frederiksberg, Denmark

PS01-037. **Functional analysis of BAK1-interacting protein 89 in plant innate immunity.** T. Halter[1], S. Mazzotta[1], S. Postel[1], C. Beuter[1], C. Buecherl[2], M. Wierzba[3], T. Nuernberger[1], C. Zipfel[4], F. Tax[3], S. de Vries[2], B. Kemmerling[1]. [1]ZMBP Tuebingen Plant Biochemistry department, [2]University Wageningen, Wageningen, The Netherlands, [3]University of Arizona, Tucson, USA, [4]The Sainsbury Laboratory, Norwich, United Kingdom

PS01-038. **Ethylene responsive AP2/ERF transcription factor MACD1 participates in phytotoxin-triggered programmed cell death.** K. Mase[1], N. Ishihama[2], H. Mori[1], H. Takahashi[3], H. Kaminaka[4], M. Kodama[4], H. Yoshioka[1]. [1]Nagoya University, Nagoya, Japan, [2]RIKEN, Kanagawa, Japan, [3]Tohoku University, Sendai, Japan, [4]Tottori University, Tottori, Japan

PS01-039. **A moss MAP kinase required for PAMP triggered immunity and defence against necrotrophic fungi.** S. Bressendorf[1], I. P. de Leon[2], J. Mundy[1], M. Petersen[1]. [1]Department of Biology, Copenhagen University, Copenhagen, Denmark, [2]Departamento de Biologa Molecular, Instituto de Investigaciones Biologicas Clemente Estable, Montevideo, Uruguay

PS01-040. **Characterization of putative *Arabidopsis thaliana* MAP-kinase substrates related to defense responses.** L. Eschen-Lippold[1], J. Loehr[1], L. Maldonado-Bonilla[1], K. Naumann[1], M. Palm-Forster[1], D. Scheel[1], J. Lee[1]. [1]Leibniz Institute of Plant Biochemistry, Halle (Saale) , Germany

PS01-041. **Identification and characterization of the novel fungal MAMP SsE1 and its RLP-based recognition complex in *Arabidopsis thaliana*.** W. Zhang[1], M. Fraiture[1], C. Zipfel[2], F. Brunner[1], A. Gust[1]. [1]Center for Plant Molecular Biology, Dept. of Plant Biochemistry, University of Tuebingen,Tuebingen, Germany, [2]The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom

PS01-042. **DAMP signalling in plant innate immunity.** P. R. Davidsson[1], K. Sims-Huopaniemi[1], H. Mikkonen[1], M. Piisila[1], T. Kariola[1], T. Palva[1]. [1]Department of Biosciences, University of Helsinki, Helsinki, Finland

PS01-043. ***In planta* identification and functional analysis of EFR complex components.** Y. Kadota[1,2], J. Sklenar[1], K. Shirasu[2], A. Jones[1], C. Zipfel[1]. [1]The Sainsbury Laboratory, UK, [2]Plant Immunity Research Group, RIKEN Plant Science Center, Japan

PS01-044. **Phosphoproteomics of MAMP-triggered immunity.** H. Matsui[1], Y. Nomura[1], K. Shirasu[2], H. Nakagami[1]. [1]Plant Proteomics Research Unit, Plant Science Center, RIKEN, Japan, [2]Plant Immunity Research Group, Plant Science Center, RIKEN, Japan

PS01-045. **The ubiquitin ligases PUB22, PUB23 and PUB24 regulate PAMP-triggered responses by targeting a component of the exocytic pathway.** M. Stegmann[1], R. G. Anderson[2], K. Ichimura[3], T. Pecenkova[4], P. Reuter[6], V. Zarsky[4], J. M. McDowell[2], K. Shirasu[5], M. Trujillo[1]. [1]Independent Junior Research Group, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany, [2]Virginia Tech, Dept. of Plant Pathology, Physiology, & Weed Science, 550 Latham Hall, Blacksburg, Va 24061-0329, United States of America, [3]Kagawa University, Faculty of Agriculture, Department of Applied Biological Science, Chair of Applied Bioresource Science, Kagawa 761-0795, Japan, [4]Institute of Experimental Botany, ASCR Rozvojova 263, Prague 6, CZ-165 02, Czech Republic, [5]RIKEN Plant Science Center, Tsurumi-ku, Yokohama 230-0045, Japan, [6]Julius-Maximilans-University of Wurzburg, Julius-von-Sachs Institute, Julius-von-

Sachs-Platz 2, D-97082 Wurzburg, Germany

PS01-046. **Artificial evolution of the NB-LRR, Rx, to enhance activation sensitivity in a broad recognition background.** C. J. Harris[1], E. Slootweg[2], L. Spiridon[3], D. C. Baulcombe[1]. [1]Department of Plant Sciences, Cambridge University, Cambridge, UK, [2]Laboratory of Nematodology, Plant Sciences Department, Wageningen University, Netherlands, [3]Institute of Biochemistry of the Romanian Academy, Bucharest 17, Romania

PS01-047. **Characterization of calcium signalling mutants in *Arabidopsis thaliana* innate immunity.** S. Ranf[1], J. Grimmer[1], D. Scheel[1], J. Lee[1]. [1]Leibniz Institute of Plant Biochemistry, Department of Stress and Developmental Biology, D-06120 Halle (Saale), Germany

PS01-048. **Receptors-like kinases go endosomal: a family picture of dynamic PRR subcellular localization in a ligand-specific manner.** F. Gervasi[1], M. Mbengue[1], S. Bartels[2], T. Boller[2], C. Zipfel[1], T. Ueda[3], S. Robatzek[1]. [1]The Sainsbury Laboratory, [2]Botanical Institute - University of Basel, [3]Laboratory of Developmental Cell Biology - Tokyo

PS01-049. **Evasion of host immune recognition of flagellin in plant and human cells by bacterial pathogens.** M. J. C. Pel[1], B. W. Bardoel[2], S. Van der Ent[1], M. F. Seidl[3], J. A. G. Van Strijp[2], C. M. J. Pieterse[1]. [1]Plant-Microbe Interactions, Utrecht University, Utrecht, The Netherlands, [2]Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands, [3]Theoretical Biology & Bioinformatics, Utrecht University, Utrecht, The Netherlands

PS01-050. **The PAMP-triggered immunity response is involved in the plant defense response to aphid attack and is suppressed by an aphid effector.** C. L. Drurey[1], D. Prince[1], S. A. Hogenhout[1]. [1]Department of Cell and Developmental Biology, The John Innes Centre, Norwich, UK

PS01-051. **Screening proteins with "VQ"motif: The quest for MAPK substrates involved in plant immunity.** P. Pecher[1], K. Kuhle[1], G. Bethke[2], S. Herklotz[1], D. Scheel[1], J. Lee[1]. [1]Leibniz Institute of Plant Biochemistry,Halle, Germany, [2]Department of Plant Biology, Microbial and Plant Genomics Institute, University of Minnesota, 1500 Gortner Avenue, St. Paul 55108, U.S.A.

PS01-052. **JAZ protein is a critical component of stomatal immunity.** N. Obulareddy[1], B. Thompson[1], M. Melotto[1]. [1]Department of Biology, University of Texas

PS01-053. **Evaluation of PAMP-triggered immunity for developing durable disease control in barley and wheat.** H. Schoonbeek[1], C. Liller[1], M. Smedley[2], E. Wallington[3], C. Zipfel[4], C. Ridout[1]. [1]Crop Genetics, John Innes Centre, Norwich, United Kingdom, [2]BRACT, John Innes Centre, Norwich, NR4 7UH, United Kingdom, [3]NIAB, Cambridge, CB3 0LE, United Kingdom, [4]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

PS01-054. **The host cell actin cytoskeleton is altered in plants infected with *Pseudomonas syringae*.** M. Shimono[1], J. L. Henty[2], K. Porter[1], W. J. Thomas[3], J. Chang[3], C. J. Staiger[2], B. Day[1]. [1]Department of Plant Pathology, Michigan State University, Michigan, USA, [2]Purdue University, IN, USA, [3]Oregon State University, OR, USA

PS01-055. **Defense-related WRKY transcription factors respond to components of the plant cell wall in *Arabidopsis*.** C. de Azevedo Souza[1,2], S. Li[1,2], S. Somerville[1,2]. [1]Energy Biosciences Institute, University of California, Berkeley, USA, [2]Department of Plant and Microbial Biology, University of California, Berkeley, USA

PS01-056. **Identification of signalling partners and internalization regulators of FLS2 by mass spectrometry.** M. Mbengue[1], H. Haweker[1], J. Sklenar[1], A. Jones[1], S. Robatzek[1]. [1]The Sainsbury Laboratory

PS01-057. **Flagellin and the role of the *Pseudomonas syringae* type III secretion system in eliciting and suppressing immune responses independent of effectors.** A. Collmer[1], S. Chakravarthy[1], H.-L. Wei[1]. [1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, USA

PS01-058. **Role of the *HaHOG1* MAP kinase in response of the conifer root and but Rot pathogen (*Heterobasidion annosum*) to osmotic and oxidative stress.** T. Raffaello[1], S. Kerio[1], F. O. Asiegbu[1]. [1]Department of Forest Sciences, University of Helsinki, Helsinki, Finland

PS01-059. **Interplay between two Arabidopsis genes, *NHR1A* and *NHR1B*, regulating stomatal defense and nonhost disease resistance against bacterial pathogens.** S. Lee[1], M. Senthil-Kumar[1], K. S. Mysore[1]. [1]The Samuel Roberts Noble Foundation, Ardmore, OK, USA

PS01-060. **Does the Arabidopsis endogenous peptide elicitor/receptor Pep/PEPR pathway act in danger sensing and signalling in plant immunity?** K. Yamada[1], M. Yamashita-Yamada[1],

K. Kanehara[1], N. Tintor[1], Y. Saijo[1]. [1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany

PS01-061. **A classification tool for calcium dependent protein kinases (CPKs) based on motif analysis.** K. R. Arthur[1], G. Valmonte[1,2], C. M. Higgins[2], R. MacDiarmid[1,3]. [1]The New Zealand Institute for Plant and Food Research Ltd, [2]School of Applied Sciences, AUT University, New Zealand, [3]School of Biological Sciences, The University of Auckland, New Zealand

PS01-062. **Molecular characterization of wound-inducible MAPK cascade in rice.** S.-J. Yoo[1], D.-H. Yang[1], B. H. Cho[1], K.-Y. Yang[1]. [1]Department of Plant Biotechnology (BK21 program), University of Chonnam, Gwang ju, Korea

PS01-063. **Different receptor systems regulate chitin signaling in *Arabidopsis* and rice.** T. Shinya[1], N. Motoyama[1], M. Hayafune[1], K. Kamiya[1], H. Shimada[1], T. Tanimoto[1], H. Kaku[1], N. Shibuya[1]. [1]Department of Life Sciences, Meiji University, Kanagawa, Japan

PS01-064. **Phosphorylation of *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defense response.** N. Ishihama[1,2], H. Adachi[2], R. Yamada[2], Y. Katou[2], M. Yoshioka[2], H. Yoshioka[2]. [1]Plant Immunity Research Group, RIKEN Plant Science Center, Yokohama, Japan, [2]Laboratory of Defense in Plant-Pathogen Interactions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

PS01-065. **Post-translational modification of WRKY transcription factors by MAPKs induces HR-like cell death.** H. Adachi[1], N. Ishihama[2], M. Yoshioka[1], Y. Katou[1], T. Yaeno[2], K. Shirasu[2], H. Yoshioka[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Japan, [2]RIKEN Plant Science Center

PS01-066. **Identification of flagellin receptor in rice involved in induction of plant immune responses.** Y. Katsuragi[1], A. Oguri[1], T. Morimoto[1], K. Kajiyama[1], H. Kajimoto[1], Y. Tanaka[1], R. Takai[1], F.-S. Che[1]. [1]Graduate School of Biosciences, Nagahama Institute of Bio-Science and Technology, Shiga,Japan

PS01-067. **An interaction-based identification of MKK3 upstream factors in *Arabidopsis*.** M. Nakamura[1], K. Takigawa[2], K. Shirasu[2], K. Ichimura[1]. [1]The agricultural department, University of Kagawa, Kagawa, Japan, [2]RIKEN Plant Science Center, Kanagawa, Japan

PS01-068. **Functional analysis of LysM motifs in rice chitin receptor CEBiP.** M. Hayafune[1], S. Arima[1], M. Kayama[1], K. Kamiya[1], T. Shinya[1], K. Okada[2], H. Yamane[2], N. Shibuya[1], H. Kaku[1]. [1]Department of Life Sciences,Meiji University,Kanagawa,Japan, [2]Biotechnology Research Center,The University of Tokyo,Tokyo,Japan

PS01-069. **Identification and localization of the NB-LRR gene family in the hot pepper genome (*Capsicum annuum*).** S.-I. Yeom[1], S. Kim[1], E. Seo[1], S.-B. Kim[1], S.-Y. Kim[1], H.-A. Lee[1], Y.-M. Kim[1], D. Choi[1]. [1]Department of Plant Science, Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea

PS01-070. **Discovery of a small peptide that can activate the plant immune system from combinatorial random peptide libraries.** M. Miyashita[1], M. Oda[1], Y. Ono[1], E. Komoda[1], H. Miyagawa[1]. [1]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PS01-071. **Arabidopsis ubiquitin ligase ATL31 which is involved in defense response ubiquitinates 14-3-3 proteins in phosphorylation-dependent manner.** S. Yasuda[1], S. Maekawa[1], T. Sato[1], J. Yamaguchi[1]. [1]Faculty of Science and Graduate School of Life Science, Hokkaido University, Sapporo, Japan

PS01-072. **Towards understanding MAPK cascade function in potato-PVY interaction.** A. Coll[1], A. Lazar[1], P. Bedina[2], G. Anderluh[2], J. Zel[1], K. Gruden[1]. [1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia, [2]Laboratory for Biosynthesis and Biotransformation, National Institute of Chemistry, Ljubljana, Slovenia

PS01-073. **Two U-box ubiquitin ligases positively contribute to MAMP-responsive MAP kinase cascade in *Arabidopsis*.** J. Hio[1], K. Ichimura[1], T. Mizoguchi[2], A. Graf[3], F. Takahashi[4], K. Shinozaki[4], K. Shirasu[4]. [1]Faculty of Agriculture, University of Kagawa, Kagawa, Japan, [2]Institute of Biological Sciences, University of Tsukuba, Japan, [3]The Sainsbury Laboratory, John Innes Centre, United Kingdom, [4]RIKEN Plant Science Center, Japan

PS01-074. **Visualisation of lateral plasma membrane segregation and phosphorylation-dependent dynamics of remorin proteins in *Arabidopsis thaliana*.** I. K. Jarsch[1], S. Konrad[1], T. Ott[1]. [1]Genetics Institute. University of Munich, Munich, Germany

PS01-075. **Towards the identification of NFBS2: a high affinity Nod Factor Binding Site in *Medicago truncatula* cell suspension cultures.** J. Fliegmann[1,2], S. Canova[3], C. Lachaud[1,2], V. Gasciolli[1,2], S. Uhlenbroich[4,5], C. Pichereaux[6], B. Lefebvre[1,2], E. A. Martinez[7], H. Driguez[7], S. Cottaz[7], S. Fort[7], F. Debellé[1,2], C. Rosenberg[1,2], D. Pitorre[1,2], C. Gough[1,2], J.-M. Beau[3], B. Vauzeilles[3], M. Rossignol[6], J. V. Cullimore[1,2], J.-J. Bono[1,2]. [1]INRA, Laboratoire des Interactions

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PS01-076. **The binding affinity to viral coat proteins determines the recognition specificity of allelic L tobamovirus resistance proteins.** K.-T. Sekine[1], R. Tomita[1], G. Atsumi[1], H. Chen[2], M. Kaido[3], N. Yamaoka[2], M. Nishiguchi[2], T. Okuno[3], K. Kobayashi[2]. [1]Iwate Biotechnology Research Center, Iwate, Japan, [2]Faculty of Agriculture, Ehime University, Ehime, Japan, [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PS01-077. **Molecular analysis of *Pia*-mediated resistance, regulated by a pair of NB-LRR proteins.** T. Fujiwara[1], Y. Kawano[1], R. Terauchi[2], T. Kawasaki[1,3], K. Shimamoto[1]. [1]Grad. Sch. of Bio. Sci, NAIST, [2]Iwate Biotech. Res. Center, [3]Fuc. of Agri. Kinki Univ.

PS01-078. **A new family of endogenous peptide elicitors conserved in Fabales and Cucurbitales.** Y. Yamaguchi[1], K. Ichikawa[1], G. Pearce[2]. [1]Research Faculty of Agriculture, Hokkaido University, [2]Lewis-Clark State College

PS01-079. **Elucidation of the defensive role of GmPep914 and Gmpep890 in soybean plant.** M. Imamura[1], Y. Yamaguchi[1]. [1]Research Faculty of Agriculture, Hokkaido University

PS01-080. **Characterising endosomal proteomes during defence responses.** W. Heard[1], J. Sklenar[1], S. Robatzek[1], A. Jones[1]. [1]The Sainsbury Laboratory

PS01-081. **Quantitative proteomics reveals dynamic changes at the plasma membrane during *Arabidopsis* immune signaling.** J. M. Elmore[1], J. Liu[1], B. Phinney[2], G. Coaker[1]. [1]Depratment of Plant Pathology, University of California at Davis, USA, [2]Genome Center Proteomics Core Facility, University of California at Davis, USA

PS01-082. **FLS2/BIK1/BAK1 association and dissociation are not sufficient to activate *Arabidopsis* immunity but FLS2 phosphorylation site Ser-938 is required.** Y. Cao[1], A. Bent[1]. [1]University of Wisconsin - Madison

PS01-083. **Heterotrimeric G-proteins participate in MAMP-triggered immunity in *Arabidopsis*.** S. A. Lawrence[1], N. K. Clay[1]. [1]Department of Molecular, Cellular Developmental Biology, Yale University, New Haven, CT

PS01-084. **Identification of two *Arabidopsis* glycosyltransferases involved in the perception of MAMPS.** T. Ceserani[1], N. K. Clay[1]. [1]Molecular Cellular and Developmental Biology, Yale University, New Haven, CT, USA

PS01-085. **N-acyl-homoserine lactone confers resistance toward biotrophic pathogens via altered activation of AtMPK6.** A. Schikora[1], S. Schenk[1], E. Stein[1], K.-H. Kogel[1]. [1]Justus Liebig University Giessen

PS01-086. **Mechanism of CDPK function in local and systemic plant innate immune responses.** T. Romeis[1], W. Schulze[2], U. Dubiella[1], H. Seybold[1], G. Durian[1], E. Kommander[1], R. van Lassig[1]. [1]Dahlem Centre of Plant Sciences, [2]Max Planck Institute of Molecular Plant Physiology, Golm, Germany

PS01-087. **Dissection of disease resistance in lettuce using RNAi.** M. Christopoulou[1], L. McHale[2], M. J. Truco[1], D. Lavelle[1], T. Wroblewski[1], O. Ochoa[1], A. Kozik[1], R. W. Michelmore[1]. [1]Genome Center, University of California-Davis, Davis, U.S.A., [2]Department of Horticulture & Crop Science, Ohio State University, Columbus OH 43210, USA

PS01-088. **An *Arabidopsis* Integrin-linked protein kinase 1 homologue is involved in stress response.** E. K. Brauer[1], S. C. Popescu[1]. [1]Plant Pathology and Plant-Microbe Biology, Cornell Univserity, Ithaca, USA

PS01-089. **Map-based cloning of RPS7, an additional resistance gene in *Arabidopsis thaliana* recognizing the *Pseudomonas syringae* effector AvrRps4.** S. B. Saucet[1], K. H. Sohn[1], J. D. G. Jones[1]. [1]The Sainsbury Laboratory, John Innes Centre, Norwich, United Kingdom

PS01-090. **Identification of novel components of the innate immunity in rice.** T. Ueba[1], M. Fujiwara[2], T. Fujiwara[1], S. Hamada[1], Y. Kawano[1], K. Shimamoto[1]. [1]Labolatory of Plant Molecular Genetics, Nara Institute of science and technology, Japan, [2]Plant Global Educational Project, Nara Institute of science and technology, Japan

PS01-091. **Chromatin-associated regulation of plant innate immunity by the Arabidopsis PHD-finger-like protein EDM2.** T. Tsuchiya[1], T. Eulgem[1]. [1]Botany and Plant Sciences, University

of California at Riverside, Riverside, USA

PS01-092. **Refining the model of R protein activation using the M flax-rust resistance protein.** E. deCourcy-Ireland[1], P. Sornaraj[1], S. J. Williams[2], R. I. Menz[1], B. Kobe[2], P. A. Anderson[1]. [1]School of Biological Sciences, Flinders University, Adelaide, South Australia, Australia, [2]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD

PS01-093. **Novel role for a CBL/CIPK signaling module and its targets in plant immunity.** Y. Pareja-Jaime[1], F. de la Torre[1], E. Gutierrez[1], O. del Pozo[1]. [1]Instituto de Bioquimica Vegetal y Fotosintesis, Universidad de Sevilla/CSIC, Seville, Spain

Symbiosis I / II

PS02-094. ***In vitro*** **synthesis of the mycelial aggregate *'shiro'* required for *'matsutake'* mushroom production between the ectomycorrhizal fungus *Tricholoma matsutake* and the arbuscular-mycorrhizal plant *Cedrela odorata* regenerated from somatic embryos.** H. Murata[1], A. Yamada[2], K. Yamamoto[2], N. Endo[2], T. Maruyama[3]. [1]Department of Applied Microbiology and Mushroom Science, Forestry and Forest Products Research Institute, [2]Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, [3]Departement of Molecular and Cell Biology, Forestry and Forest Products Research Institute

PS02-095. **Inconsistent role of rhizobial ACC deaminase in the root-nodule symbiosis.** V. Murset[1], G. Pessi[2], H. Hennecke[1]. [1]Institute of Microbiology, ETH, Zurich, Switzerland, [2]Institute of Plant Biology, University of Zurich, Zurich, Switzerland

PS02-096. **Nodule bacteria of mungbean (*Vigna radiata*) growing in the Central Asia.** K. T. Yadgarov[1], M. Abzalov[1], Z. A. Khojiev[1], B. R. Umarov[2], S. S. Burikhanov[2], R. M. Usmanov[1]. [1]Institute of Genetics and Experimental Biology of Plants, [2]Institute of Microbiology AS RUz

PS02-097. **Identification of root-nodule bacteria isolated from desert zones of Central Asia.** B. R. Umarov[1]. [1]Institute of Microbiology AS RUz

PS02-098. **A SNARE protein expressed in vascular tissue affects symbiotic nitrogen fixation in *Lotus japonicus* nodules.** T. Hakoyama[1,2], R. Oi[1], K. Hazuma[1], E. Suga[1], Y. Adachi[1], M. Kobayashi[1], R. Akai[1], S. Sato[3], E. Fukai[3], S. Tabata[3], S. Shibata[2], G.-J. Wu[2], Y. Hase[4], A. Tanaka[4], M. Kawaguchi[5], H. Kouchi[2], Y. Umehara[2], N. Suganuma[1]. [1]Department of Life Science, Aichi University of Education, [2]National Institue of Agrobiological Sciences, [3]Kazusa DNA Research Institute, [4]Japan Atomic Energy Agency, [5]National Institute for Basic Biology

PS02-099. **Lon protease of *Azorhizobium caulinodans* ORS571 is required for the suppression of *reb* genes expression.** A. Nakajima[1], L. L. Siarot[1], S. Tsukada[1], T. Ogawa[2], T. Aono[1], H. Oyaizu[1]. [1]Biotechnology Research Center, The University of Tokyo, Tokyo, Japan, [2]Department of Biotechnology, Graduate School of Agricultural and Life Science, The University of Tokyo

PS02-100. **Effect of external nitrogen concentration and light intensity on nodulation, nitrogen fixation and growth of cowpea (*Vigna unguiculata* L. Walp.).** P. S. Sarr[1], S. Fujimoto[2], T. Yamakawa[3]. [1]Center for African Area Studies, Kyoto University, Kyoto, Japan, [2]Graduate School of Bioresources and Bioenvironmental Sciences, Kyushu University, [3]Department of Bioresource and Biotechnology, Faculty of Agriculture, Kyushu University

PS02-101. **The rice NPC protein defines a new class of potential transporter with an essential role during AM symbiosis.** M. Nadal[1], R. Sawers[2], C. Gutjahr[3], J. Arbuckle[5], G. An[4], K. An[4], U. Paszkowski[1]. [1]Department of Plant Molecular Biology, University of Lausanne, Lausanne, Switzerland, [2]Laboratorio Nacional de Genómica para la Biodiversidad, Irapuato, Mexico, [3]Institute of Genetics, University of Munich, Martinsried, Germany, [4]National Research Laboratory of Plant Functional Genomics, Pohang University of Science and Technology,Pohang Korea, [5]Pioneer Hi-bred International Inc., Des Moines, Iowa, USA

PS02-102. **Evaluation of effective *Bradyrhizobium* strains from Myanmar and co-inoculation with endophytic *Streptomyces* sp.** K. M. Soe[1], T. Yamakawa[2]. [1]Graduate School of Biosciences and Bioresources, Faculty of Agriculture, Kyushu University, Japan, [2]Division of Molecular Biosciences, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 812-8581

PS02-103. ***Bradyrhizobium japonicum*** **character predicted from genomic comparison of two strains.** T. Kaneko[1], N. Uchiike[1], H. Maita[2], H. Hirakawa[2], K. Minamisawa[3], A. Watanabe[2], S. Sato[2]. [1]Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan, [2]Kazusa DNA Research Institute, [3]Graduate School of Life Sciences, Tohoku University

PS02-104. **Arbuscular collapse regulates carbon release by hosts in mycorrhizal symbiosis.** Y. Kobae[1], C. Gutjahr[2,3], U. Paszkowski[2], S. Hata[1]. [1]Graduate School of Agricultural and Life

Sciences, University of Tokyo, Tokyo, Japan, [2]Department of Plant Molecular Biology, University of Lausanne, 1015 Lausanne, Switzerland, [3]University of Munich (LMU), Faculty of Biology, Institute of Genetics, Grosshaderner Str. 2-4, D-82152 Martinsried, Germany

PS02-105. **Analysis of common symbiosis system reveals infection mechanism of arbuscular mycorrhizal fungi in *Lotus japonicus*.** N. Takeda[1,2,3], T. Maekawa[2], M. Hayashi[3], M. Parniske[2], M. Kawaguchi[1]. [1]National Instutite for Basic Biology/SOKENDAI, Aich, Japan, [2]LMU Munich, Munich, Germany, [3]National Institute of Agrobiological Sciences, Tsukuba, Japan

PS02-106. **Identification of a novel *nodule inception (nin)* mutant, *daphne* that displays a non-nodulation but dramatically increased number of infection threads.** E. Yoro[1,2], T. Suzaki[1,2], M. Kawaguchi[1,2]. [1]Division of Symbiotic Systems, National Institute for Basic Biology, Aichi, Japan, [2]Department of Basic Biology, The Graduate University for Advanced Studies, Kanagawa, Japan

PS02-107. ***Lotus japonicus AMP1* and *HAR1* act synergistically to regulate root architecture.** C. S. Kim[1,2], M. Held[1,2], T. Suzaki[3], B. Karas[1,2], S. Sato[4], S. Tabata[3], M. Kawaguchi[3], K. Szczyglowski[1,2]. [1]Southern Crop Protection and Food Research, Agriculture and Agri-Food Canada, London, Canada, [2]Department of Biology, University of Western Ontario, London, ON, N6A5B7, Canada, [3]Division of Symbiotic Systems, National Institute for Basic Biology, Aichi 444-8585, Japan, [4]Kazusa DNA Research Institute, Kisarazu, Chiba 292-0812, Japan

PS02-108. **The root regulator *TOO MUCH LOVE* functions in the *CLE-RS1*/*RS2*-mediated long distance control of nodulation.** M. Takahara[1,2], S. Magori[3], S. Okamoto[2], C. Yoshida[4], K. Yano[2], S. Sato[5], S. Tabata[5], K. Yamaguchi[2], S. Shigenobu[1,2], N. Takeda[1,2], T. Suzaki[1,2], M. Kawaguchi[1,2]. [1]Department of Basic Biology in the School of Life Science of the Graduate University for Advanced Studies, Aichi, Japan, [2]National Institute for Basic Biology, Aichi, Japan, [3]Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY, USA, [4]Department of Biological Science, Graduate School of Science,The University of Tokyo, Tokyo, Japan, [5]Kazusa DNA Research Institute, Chiba, Japan

PS02-109. **Mutation of class 1 hemoglobin affects the infection of *Mesorhizobium loti* to its host plant *Lotus japonicus*.** T. Kado[1], K. Osuki[1], K. Kucho[1], M. Abe[1], S. Higashi[1], T. Uchiumi[1]. [1]Department of Chemistry and Bioscience, Kagoshima University, Kagoshima, Japan

PS02-110. **Localization of polyphosphate in arbuscular mycorrhizal fungus colonizing in *Lotus japonicus*.** K. Saito[1], Y. Osada[1], K. Nakiri[1], A. Nishimura[1], C. Matsumoto[1], M. Saito[2], T. Ezawa[3]. [1]Faculty of Agriculture, Shinshu University, Nagano, Japan, [2]Graduate School of Agricultural Science, Tohoku University, Miyagi, Japan, [3]Graduate School of Agriculture, Hokkaido University, Hokkaido, Japan

PS02-111. **Expression analysis of SWEET transporters in *Lotus japonicus*.** Y. Saida[1], A. Sugiyama[1], K. Takanashi[1], K. Yazaki[1]. [1]RISH, Kyoto University, Japan

PS02-112. **A MATE-type transporter responsible for iron supply to nodule infection zone of *Lotus japonicus*.** K. Takanashi[1], K. Yokosho[2], H. Takahashi[3], K. Saeki[4], A. Sugiyama[1], S. Sato[5], S. Tabata[5], M. Nakazono[3], J. F. Ma[2], K. Yazaki[1]. [1]Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Japan, [2]Research Institute for Bioresources, Okayama University, Kurashiki, Japan, [3]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [4]Department of Biological Sciences, Faculty of Science, Nara Women's University, Nara, Japan, [5]Kazusa DNA Research Institute, Kisarazu, Japan

PS02-113. **KLAVIER is a receptor-like kinase necessary for long-distance negative regulation of nodulation mediated by CLE-RS1/2-signaling in *Lotus japonicus*.** H. Miyazawa[1], T. Suzaki[1,2], T. Sakai[3], M. Kawaguchi[1,2]. [1]Division of Symbiotic Systems, National Institute for Basic Biology, Aichi, Japan, [2]Department of Basic Biology, School of Life Science, Graduate University for Advanced Studies (SOKENDAI), [3]Graduate School of Science and Technology, Niigata University

PS02-114. **Soybean phosphate transporter gene *GmPT7* is expressed in mycorrhizas and senescent leaves.** Y. Inoue[1], Y. Kobae[2], S. Takai[1], Y. Tamura[1], A. Hirose[3], K. Komatsu[3], M. Ishimoto[3,4], S. Hata[1]. [1]Laboratory of Crop Science, Graduate School of Bioagricultual Sciences, Nagoya University, Aichi 464-8601, Japan, [2]Laboratory of Plant Nutrition and Fertilizers, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan, [3]National Agricultural Research Center for Hokkaido Region, Hokkaido 062-8555, Japan,

[4]National Institute of Agrobiological Sciences, Ibaraki 305-8602, Japan

PS02-115. **Differential expression of arbuscular mycorrhiza-inducible acyltransferase and esterase genes of rice *(Oryza sativa)*.** T. Sisaphaithong[1], Y. Kobae[2], S. Hata[1]. [1]Laboratory of Crop Science, Graduate School of Bioagricultural Sciences, Nagoya University, Aichi 464-860, Japan, [2]Laboratory of Plant Nutrition and Fertilizers, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan

PS02-116. **Non-redundant control of rice arbuscular mycorrhizal symbiosis by two phosphate transporters.** S.-Y. Yang[1], I. Jakobsen[2], D. Rentsch[3], H. Hirochika[4], V. Sundaresan[5], N. Salamin[6], S. Heuer[7], J. Gheyselinck[1], U. Paszkowski[1]. [1]Department of Plant Molecular Biology, University of Lausanne, CH-1015 Lausanne, Switzerland., [2]Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-4000 Roskilde, Denmark., [3]University of Bern, Institute of Plant Sciences, CH-3013, Switzerland, [4]National Institute of Agrobiological Sciences, Agronomics Research Center, Tsukuba, Ibaraki 305-8602, Japan, [5]University of California Davis, Department of Plant Biology and Plant Sciences, Davis, CA 95616, USA, [6]University of Lausanne, Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland, [7]Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, DAPO box 7777, Metro Manila, Philippines

PS02-117. **Deciphering the ethylene-signaling pathway during early symbiosis in *Medicago truncatula*.** E. Larrainzar[1], A. Greenspan[1], J. Baek[1], B. K. Riely[1], H.-J. Hwang[2], M. Oh[2], S.-C. Kim[3], J.-H. Mun[2], D. R. Cook[1]. [1]Department of Plant Pathology, University of California-Davis, Davis, USA, [2]Department of Agricultural Biotechnology, National Academy of Agricultural Science, Rural Development Administration, 150 Suin-ro, Gwonseon-gu, Suwon 441-707, Korea, [3]Korean Bioinformation Center, KRIBB, Yuseong-gu, Daejeon, 305-806, Korea

PS02-118. **Rhizobial infection decides nodule identity.** D. Guan[1], N. Stacey[1], G. E. D. Oldroyd[1], J. D. Murray[1]. [1]John Innes Centre

PS02-119. **Analysis of flavonoid secretion from the root of hydroponic culture of soybean.** K. Yamashita[1], A. Sugiyama[1], K. Takanashi[1], K. Yazaki[1]. [1]Research Institute for Sustainable Humanosphere, University of Kyoto, Kyoto, Japan

PS02-120. **Uncovering the infectome: single-cell type transcriptomic studies of *Medicago truncatula* root hairs during *Sinorhizobium meliloti* infection reveals new common symbiotic genes.** A. Breakspear[1], D. Guan[1], C. Liu[1], N. Stacey[1], C. Rogers[1], J. D. Murray[1]. [1]John Innes Centre, Norwich, United Kingdom

PS02-121. **The endophyte *Epichloe festucae* requires *velvet* for a successful interaction with its host grass.** D. Fleetwood[1,2], C. Voisey[1], W. Simpson[1], W. Mace[1], M. Rahnama[2], S. Lott[1,2], R. Johnson[1]. [1]Forage Improvement, AgResearch, Palmerston North, New Zealand, [2]School of Biological Sciences, University of Auckland, New Zealand

PS02-122. **Identification of novel arbuscular mycorrhizal-specific genes regulated by gain-of-function CCaMK, a key regulator of endosymbiosis.** H. Imaizumi-Anraku[1], M. Nagae[1], Y. Shimoda[1], N. Takeda[2], M. Hayashi[1]. [1]National Institute of Agrobiological Sciences (NIAS), [2]National Institute for Basic Biology

PS02-123. **RNA-seq analysis of root nodules and arbuscular mycorrhiza in *Lotus japonicus* and *de novo* transcriptome assembly of *Glomus intraradices*.** Y. Handa[1], N. Takeda[1], Y. Suzuki[3], M. Kawaguchi[1], K. Saito[2]. [1]Division of Symbiotic Systems,National Institute for Basic Biology, Aichi, Japan, [2]Faculty of Agriculture, Shinshu University, Nagano, Japan, [3]Department of Medical Genome Sciences, Graduate School of Frontier Sciences, the University of Tokyo, Japan

PS02-124. **Auxotrophic and anaplerotic amino acid metabolism in *Mesorhizobium loti*.** S. Tajima[1], M. Nomura[1], N. Thapanapongworakul[2], A. Enoki[1], H. Matsuura[1]. [1]Dept. Applied Life Sci., Kagawa University, Japan, [2]Dept. Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, 50200 Chiang Mai, Thailand

PS02-125. **Novel arbuscular mycorrhiza-inducible phosphate transporters of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*).** S. Hata[1], T. Sisaphaithong[1]. [1]Laboratory of Crop Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

PS02-126. **Study of vesicle trafficking in *Lotus japonicus* nodules.** M. Nomura[1], T. Miyoshi[1], H. Yamasaki[1], A. Sogawa[1], S. Chungopast[1], K. Yokota[2], M. Hayashi[2], S. Tajima[1]. [1]Faculty of Agriculture Kagawa University, Kagawa, Japan, [2]NIAS, Tsukuba, Japan

PS02-127. **Comparative genome analysis of *Mesorhizobium loti* strains.** H. Maita[1,2], H. Hirakawa[1], Y. Nakamura[1,3], T. Kaneko[4], S. Tabata[1], K. Saeki[5], S. Sato[1,2]. [1]Lab. of Applied Plant Genomics, Kazusa DNA Res. Inst., [2]Graduate School of Life Sciences, Tohoku Univ., [3]Center for Info.

Biol. and DDBJ, National Inst. of Genetics, [4]Faculty of Engineering, Kyoto Sangyo Univ., [5]Faculty of Science, Nara Women's Univ.

PS02-128. **Does iron influence the nature of the symbiotic interaction of a fungus with its host grass?** N. T. Forester[1,2], G. A. Lane[1], I. L. Lamont[2], L. J. Johnson[1]. [1]Agresearch Ltd, Palmerston North, New Zealand, [2]University of Otago, Dunedin, New Zealand

PS02-129. **The expression of defense-related genes is attenuated by symbiotic signal cascades.** T. Nakagawa[1], H. Kaku[1], H. Kouchi[2], N. Shibuya[1]. [1]Department of Life Sciences, Faculty of Agriculture, Meiji University, Kawasaki, Japan, [2]NIAS

PS02-130. **Gene expression profiling of *Epichloe* endophytes in progenitor versus modern cereals.** L. J. Johnson[1], M. Gagic[1], W. Simpson[1], A. Khan[1], C. Voisey[1], R. Johnson[1]. [1]AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand

PS02-131. **Synthesis and symbiosis-related gene-inducing activity of Myc-LCOs and their *N*-acyl chain-modified derivatives.** K. Akiyama[1], C. Kawahara[1], H. Hayashi[1]. [1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan

PS02-132. **Phagocytic incorporation of PCC6803 cells in *Paramacium bursaria* and RAW264.7 cells.** A. Noriyasu[1], S. Mochizuki[1], K. Sakurai[1], T. Kawano[1]. [1]Graduate School of Environmental Engineering, The University of Kitakyushu, Fukuoka, Japan, [2]Shinichi Mochizuki, [3]Kazuo Sakurai, [4]Tomonori Kawano

PS02-133. **Role of vitamin B6 metabolic pathway in symbiotic root nodules of *Lotus japonicus*.** A. Tominaga[1,2], A. Ide[2], T. Yagi[3], S. Iwamoto[3], S. Arima[1,2], A. Suzuki[1,2]. [1]United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan, [2]Department of Environmental Sciences, Faculty of Agriculture, Saga University, Saga, Japan, [3]Department of Bioresources Science, Faculty of Agriculture, Kochi University, Kochi, Japan

PS02-134. **Development of tools for the biochemical characterization of the symbiotic receptor-like kinase DMI2.** B. K. Riely[1], E. Larrainzar[1], J.-H. Mun[2], E. Gil-Quintana[3], E. M. Gonzalez[3], D. Tricoli[4], H.-J. Yu[5], D. R. Cook[1]. [1]Department of Plant Pathology, University of California-Davis, Davis, CA, U.S.A., [2]Department of Agricultural Biotechnology, National Academy of Agricultural Science, Rural Development Administration, Gwonseon-gu, Suwon, Korea, [3]Departamento de Ciencias del Medio Natural, Universidad Publica de Navarra, Pamplona, Navarra, Spain, [4]Ralph M. Parsons Foundation Plant Transformation Facility, University of California-Davis, Davis, CA, U.S.A., [5]Department of Life Sciences, The Catholic University of Korea, Wonmi-gu, Bucheon, Korea

PS02-135. **Characterization of NO-inducing lipid A from *Mesorhizobium loti* lipopolysaccharide.** M. Hashimoto[1], Y. Tanishita[1], Y. Suda[1], E. Murakami[2], M. Nagata[2], K. Kucho[2], M. Abe[2], T. Uchiumi[2]. [1]Deptepartment of Chemistry, Biotechnology, and Chemical Engineering, Kagoshima University, [2]Department of Chemistry & Bioscience, Kagoshima University

PS02-136. **New regulatory peptides that that affect root nodule formation and lateral root initiation in *Medicago truncatula*.** N. Imin[1], N. A. M. Radzman[1], E. H. Scholl[2], P. DiGennaro[2], M. Oakes[1], D. M. Bird[2,3], M. A. Djordjevic[1]. [1]Plant Science Division, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, ACT, Australia., [2]Department of Plant Pathology , North Carolina State University, Raleigh, NC 27695, USA, [3]Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695, USA

PS02-137. **The use of phosphate-solubilizing rhizobacteria as biofertilizer to enhance soybean plant growth.** S. Meliah[1], N. R. Sari[1], A. T. Wahyudi[1], A. A. Nawangsih[2], E. Husen[3]. [1]Departement of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia, [2]Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Indonesia, [3]Indonesian Soil Research Institute, Indonesia

PS02-138. **Symbiotic nitrogen fixation triggers global changes in bacterial and plant sulphur metabolism.** C. Kalloniati[1], P. Krompas[1], G. Karalias[1], C. Herschbach[2], H. Rennenberg[2], E. Flemetakis[1]. [1]Department of Agricultural Biotechnology, Agricultural University of Athens, Athens, Greece, [2]Institute of Forest Botany and Tree Physiology, Chair of Tree Physiology, University of Freiburg, Georges-Kohler-Allee 53, 79110 Freiburg, Germany

PS02-139. **Characterization of transcription factors of *Medicago truncatula* involved in the arbuscular mycorrhizal symbiosis.** J. Teply[1], A. Reinert[1], E. Devers[1], F. Krajinski[1]. [1]Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

Pathogenic fungi

PS03-140. **Identification of *Fusarium graminearum* secreted proteins involved in the interaction with barley and wheat.** F. Yang[1]. [1]Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark

PS03-141. **Genetic diversity and PCR-based identification of potential fumonisin-producing *Fusarium verticillioides* isolates infecting corn in the Philippines.** N. J. F. Magculia[1], C. J. R. Cumagun[2]. [1]International Rice Research Institute, Los Banos, Laguna, Philippines, [2]University of the Philippines Los Banos, College, Laguna, Philippines

PS03-142. **Transient and multivariate system for transformation of a fungal plant pathogen, *Rosellinia necatrix*, using autonomously replicating vectors.** T. Shimizu[1], T. Ito[1], S. Kanematsu[1]. [1]Apple Research Station, Institute of Fruit Tree Science, National Agriculture and Food Research Organization, Iwate, Japan

PS03-143. **Arabidopsis GNOM ARF-GEF and barley ARFA1b/1c GTPase link multivesicular bodies to syntaxin-regulated penetration resistance.** H. Thordal-Christensen[1], M. E. Nielsen[1], H. Boehlenius[1]. [1]Dept. of Agriculture and Ecology, University of Copenhagen, Denmark

PS03-144. **Isolation of plant and powdery mildew components defining and controlling formation of the extrahaustorial membrane.** M. Kwaaitaal[1], G. Aguilar[1], S. Hanisch[1], H. Thordal-Christensen[1]. [1]Defence Genetics group, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Denmark

PS03-145. **A pH-responsive transcriptional factor is involved in the entry mode selection of *Colletotrichum orbiculare* at wounded sites of Arabidopsis leaves.** K. Yoshino[1], T. Okuno[1], Y. Takano[1]. [1]Division of Applied Bioscience, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PS03-146. **Tracking of esca causal agents, *Phaemoniella chlamydospora* and *Phaeoacremonium aleophilum*, in young vine plants.** J. Pouzoulet[1], R. Pierron[1,2], S. Compant[2], N. Mailhac[1], A. Jacques[1]. [1]Université de Toulouse, Equipe Vins Viticulture et OEnologie, Département des sciences agronomiques et agroalimentaires, INP-EI Purpan, [2]Université de Toulouse, LGC UMR 5503 (CNRS/UPS/INPT), DeptBIOSYM, INP-ENSAT, 1 avenue de l Agrobiopole, 31326 Castanet-Tolosan, France

PS03-147. **A possible alternative target of Roxithromycin in fungi.** A. Ishii[1], M. Kumasaka[1], Y. Koizumi[1], T. Kamakura[1]. [1]Faculty of Science and Technology, Tokyo University of Science, tiba

PS03-148. **A Plant-microbe interaction between strawberry cultivar Ecchiesu-138 and the causal *Alternaria* pathogen is homologous with that between cultivar Morioka-16 and the strawberry pathotype of *A. alternata*.** M. Yamamoto[1], M. Asano[1]. [1]Faculty of Agriculture, Okayama University, Okayama, Japan

PS03-149. **Switching between pathogenicity and saprophytic phase in *Heterobasidion annosum*.** H.-C. Kuo[1], J. P. T. Valkonen[2], F. O. Asiegbu[1], Y. H. Lee[1,3]. [1]Department of Forest Sciences, University of Helsinki, Helsinki, Finland, [2]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland, [3]Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

PS03-150. **Global expression profiling of transcription factor genes provides new insights on pathogenicity and stress responses in the rice blast fungus.** S.-Y. Park[1], J. Choi[1], S.-E. Lim[1], G. Lee[1], J. Park[1], Y. Kim[2], S. Kong[1], S. Kim[1], H.-S. Rho[1], J. Jeon[1], M.-H. Chi[1], S. Kim[1], C. H. Khang[3], S. Kang[4], Y.-H. Lee[1]. [1]Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, [2]Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea, [3]Dept. of Plant Biology, University of Georgia, Athens, GA30602, USA, [4]Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA

PS03-151. **Investigating the role of deduced polarity establishment factors, CoCDC42 and CoBEM1, in infectious morphogenesis of *Colletotrichum orbiculare*.** T. Nomura[1], M. Kawashimo[1], D. Takemoto[2], Y. Kubo[1], G. Tsuji[1]. [1]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan, [2]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

PS03-152. ***MoERR1* encoding an ER retention protein receptor is required for asexual development and pathogenicity in the rice blast fungus.** J. Goh[1], M. Yi[1,2], S.-Y. Park[1]. [1]Department of Agricultural Biotechnology, Center for Fungal Resources, Center for Fungal Pathogenesis,

Agriculture and Life Science, Seoul National University, Seoul, Korea, [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506

PS03-153. **Cellular dynamics of *Magnaporthe oryzae* during infection process both on hydrophobic (leaf) and hydrophilic (root) surfaces.** K. Inoue[1,2], P. Park[1,2], K. Ikeda[1,2]. [1]Laboratory of Stress Cytology, Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]Bio-oriented Technology Research Advancement Institution

PS03-154. **A putative lipid phosphate phosphatase is required for defense responses in *Arabidopsis thaliana* to adapted and non-adapted pathogens.** S. Oide[1], V. Montiel[1], H. Peele[1], J. Lindberg Yilmaz[2], M. Persson[3], N. Guan[1], C. Dixelius[1]. [1]Department of Plant biology and Forest Genetics, Swedish University of Agriculture, [2]ScanBiRes AB, Jenny Lindberg Yilmaz, 2, [3]Department of Botany, Stockholm University, 3

PS03-155. **Putative components of a protein complex for processing of ACR-toxin Sensitivity gene (*ACRS*) mRNA.** K. Ohtani[1], S. Yasuda[1], S. Nishimura[1], C. Miyake[1], S. Tatano[1], Y. Ono[1], S. Nishida[1], Y. Tada[1], K. Ichimura[1], K. Gomi[1], K. Akimitsu[1]. [1]Faculty of Agriculture, Kagawa University, Kagawa, Japan

PS03-156. **Dissection of genes involved in trichothecene biosynthesis and virulence in *Fusarium graminearum*.** Y.-C. Liao[1,2,3], H.-P. Li[3,4], J.-B. Zhang[1,2], T. Huang[1,4], X.-S. Song[1], J.-H. Wang[1,2], B. Song[1], X.-Y. Wang[1], X.-M. Du[1], B. Qu[1,2]. [1]Molecular Biotechnology Laboratory of Triticeae Crops, Huazhong Agricultural University, Wuhan, China, [2]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China, [3]National Center of Plant Gene Research (Wuhan), Wuhan 430070, PR China, [4]College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

PS03-157. **Functional analysis of germ tube expressing cDNA library of *Magnaporthe oryzae*.** K. Sasaki[1], Y. Koizumi[1], K. Amano[1], T. Kamakura[1]. [1]Department of Applied Biological Science, Tokyo University of Science, Chiba, Japan

PS03-158. **Transcriptional regulatory circuits necessary for appressorium-mediate plant infection by *M. oryzae*.** M. Oses- Ruiz[1], D. Soanes[1], N. J. Talbot[1]. [1]School of Biosciences, University of Exeter, Exeter, UK

PS03-159. **Transcriptome analysis of six wheat leaf rust races.** M. A. Bruce[1,2], K. Neugebauer[2], S. Wang[2], E. Akhunov[2], J. Fellers[1]. [1]USDA-ARS, HWWGRU, Manhattan, Kansas, United States of America, [2]Kansas State University, Department of Plant Pathology, Manhattan, Kansas, United States of America

PS03-160. **Transcriptional factor(s) and the regulatory region on the 5'-upstream of the *CBP1* gene specifically expressed during appressorium differentiation of *Magnaporthe oryzae*.** S. Harashima[1], K. Kusunoki[1], K. Saitoh[1], K. Izumikawa[1], M. Takeuchi[1], T. Kamakura[2], T. Arie[1], T. Teraoka[1]. [1]Tokyo University of Agriculture and Technology, [2]Faculty of Science and Technology, Tokyo University of Science

PS03-161. **Transcriptional changes mediated by chitosan in *Colletotrichum gloeosporioides*.** E. L. Ortiz-Vazquez[1], D. Chan-Rodriguez[1], G. Lizama-Uc[1], G. Pacheco-Trejo[1], J. Ramon-Sierra[1]. [1]Division de Estudios de Posgrado e Investigacion, Instituto Tecnologico de Merida

PS03-162. **Genome rearrangements abolishing the ability of *Rosellinia necatrix megabirnavirus1* to confer hypovirulence to the white root rot fungus.** S. Kanematsu[1], T. Shimizu[1], H. Yaegashi[1], A. Sasaki[1], T. Ito[1], N. Suzuki[2]. [1]Institute of Fruit Tree Science, NARO, [2]Institute of Plant Science and Resources, Okayama University

PS03-163. **Characterization of *CoIRA1* of *Colletotrichum orbiculare*, required for infection-related morphogenesis and pathogenicity.** K. Harata[1], Y. Kubo[1]. [1]Laboratory of Plant Pathology,Graduate school of Life and Enviromental science, Kyoto Prefectural University, Kyoto,Japan

PS03-164. **Functional characterization of genes encoding forkhead transcription factors in *Magnaporthe oryzae*.** J. Park[1], S.-Y. Park[1], S. Kong[1], J. Park[1], Y.-H. Lee[1]. [1]Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

PS03-165. ***Colletotrichum orbiculare CoBUB2*, the homolog of *Saccharomyces cerevisiae BUB2*, is involved in proper nuclear distribution, morphogenesis, and pathogenesis.** F. Fukada[1], A. Sakaguchi[2], Y. Kubo[1]. [1]Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto Prefectual University, [2]Present address, National Institute of Agrobiological Sciences

PS03-166. **Functional analysis of the bZIP transcription factor family in *Magnaporthe oryzae*.** S. Kong[1], S.-Y. Park[1], Y.-H. Lee[1]. [1]Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

PS03-167. **Identity and distribution of ORFs from non-tox common regions of ACT- and AF-toxin Tox chromosomes among various isolates of *Alternaria alternata*.** Y. Mizobuchi[1,2,3], K. Ohtani[1], Y. Izumi[1], Y. Miyamoto[1], A. Masunaka[1], T. Fukumoto[1], K. Gomi[1], Y. Tada[1], K. Ichimura[1], T. Tsuge[2], M. Yamamoto[3], K. Akimitsu[1]. [1]Faculty of Agriculture, Kagawa University, [2]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan, [3]College of Agriculture, Okayama University, Okayama 700-8530, Japan

PS03-168. **Isolation and characterization of soil microorganisms with anti-*Ganoderma* properties.** P. C. Loh[1], K. H. Chiang[1], H. L. Wong[2]. [1]Dept. of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Perak, Malaysia, [2]Dept. of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia

PS03-169. **Genetic studies of the sugarcane smut fungus *Sporisorium scitamineum*.** G. V. Reis[1], F. S. R. Nunes[1], D. P. Longatto[1], A. Palhares[1], L. C. B. Carvalho[1], S. A. C. Souza[2], M. L. C. Vieira[1], L. E. A. Camargo[3], C. B. Monteiro-Vitorello[1]. [1]Department of Genetics, University of Sao Paulo, Sao Paulo, Brazil, [2]Centro de Cana, Instituto Agronomico de Campinas, Sao Paulo, Brazil, [3]Department of Phytopathology, University of Sao Paulo, Sao Paulo, Brazil

PS03-170. **De novo partial genome assembly of the biotrophic eucalyptus rust pathogen *Puccinia psidii*.** M. C. Quecine[1], D. H. Moon[1], L. M. Franceschini[1], A. Bini[1], T. F. Leite[1], M. T. V. Labate[1], C. A. Labate[1]. [1]Department of Genetic, University of Sao Paulo, Piracicaba-SP, Brazil

PS03-171. **Roles of rice transcription factor OsWRKY76 in response to the rice blast fungus.** N. Yokotani[1], Y. Sato[1], S. Tanabe[1], T. Chujo[2], T. Shimizu[2], K. Okada[2], H. Yamane[2,3], M. Shimono[1], S. Sugano[1], H. Takatsuji[1], H. Kaku[4], Y. Nishizawa[1], E. Minami[1]. [1]National Institute of Agrobiological Sciences, [2]Tokyo University, [3]Teikyo University, [4]Sakata Seed Corporation

PS03-172. **Novel MAP kinase signaling cascade in *Arabidopsis* resistance to mycotoxigenic fungi.** T. Asano[1], T. Nishiuchi[1]. [1]Advanced Science Research Center, Kanazawa University, Ishikawa, Japan

PS03-173. **Characterization of isolates of *Alternaria* spp. recovered from apple in Italy.** F. Roondo[1], B. M. Pryor[1], A. Brunelli[2]. [1]Plant Sciences Department, University of Arizona, Tucson, USA, [2]University of Bologna, DiProVal department, Bologna Italy

PS03-174. **Genome-wide analysis of Pox genes in fungi.** J. Choi[1], N. Détry[2]. [1]Fungal Bioinformatics Laboratory, Seoul National University, Seoul, Korea, [2]Department of Forest Science, University of Helsinki, Helsinki, Finland

PS03-175. **TranscriptomeS of *Botrytis cinerea*.** A. Simon[1], J. Kelloniemi[2], A. Cimerman[1], B. Dalmais[1], G. Morgant[1], J. Schumacher[3], J.-M. Pradier[1], P. Le Pecheur[1], J. Roudet[4], M. Fermaud[4], B. Tudzynski[3], P. Tudzynski[3], B. Poinssot[2], M. Viaud[1]. [1]INRA, [2]INRA- U. de Bourgogne, Dijon, France, [3]U. of Muenster, Germany, [4]INRA, Bordeaux, France

Plant-oomycete / fungal interactions

PS04-176. **The trans-Golgi network/early endosome is a critical endomembrane organelle for the execution of plant stress responses.** Y. Gu[1], R. Innes[1]. [1]Department of Biology, Indiana University, Bloomington, USA

PS04-177. **The use of the soils fungus *Penicillium canescens* in the increase the harvest to Soybean plants.** K. M. Khamidova[1], B. R. Umarov[1]. [1]Institute of Microbiology AS RUz

PS04-178. **A barley RAC/ROP interacting ROP binding kinase (HvRBK1) influences microtubule stability and is involved in pathogen response to the barley powdery mildew fungus.** T. Reiner[1], C. Huesmann[1], C. Hoefle[1], J. Preuss[1], M. E. Jurca[2], M. Domoki[2], A. Feher[2], R. Hueckelhoven[1]. [1]Lehrstuhl fuer Phytopathologie, Technische Universitaet Muenchen, Freising-Weihenstephan, Germany, [2]Laboratory of Functional Cell Biology, Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, P.O. Box 521, Temesvari krt. 62, H-6726 Szeged, Hungary

PS04-179. **Secretion of effector proteins in rice blast fungus *Magnaporthe oryzae*.** Y. K. Gupta[1], Y. Dagdas[1], M. C. Giraldo[2], H. Saitoh[3], R. Terauchi[3], B. Valent[2], N. J. Talbot[1]. [1]School of Biosciences, University of Exeter, EX4 4QD, UK, [2]Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA, [3]Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003 Japan

PS04-180. **Surface-mediated response to elicitins is providing a novel layer of resistance to *Phytophthora infestans* in potato.** J. Du[1], G. Bijsterbosch[1], E. Jacobsen[1], R. G. F. Visser[1], V. G. A. A. Vleeshouwers[1]. [1]Wageningen UR Plant Breeding, Wageningen University, Wageningen, The Netherlands

PS04-181. **Identification of a hidden resistance gene in tetraploid wheat using laboratory strains of *Magnaporthe oryzae* produced by backcrosses.** C. J. R. Cumagun[1], V. V. Anh[2], T. T. P. Vy[2], Y. Tosa[2]. [1]Crop Protection Cluster, College of Agriculture, University of the Philippines Los Banos, College, Laguna, Philippines, [2]Laboratory of Plant Pathology, Graduate School of Agricultural Sciences, Kobe University, Nada, Kobe 657-8501, Japan

PS04-182. **Expression of the late blight resistance gene *Rpi-phu1* after the pathogen challenge.** M. Swiatek[1], I. Tomczynska[1], M. Chmielarz[1], J. Sliwka[1]. [1]Plant Breeding and Acclimatization Institute - National Research Institute, Mlochow Research Centre, Mlochow, Poland

PS04-183. **Transcriptome analysis of hexaploid wheat in the early stages of floral colonisation by the ergot fungus *Claviceps purpurea*.** A. Gordon[1], P. Grant[2], G. L. A. Barker[3], V. Fanstone[1], R. Bayles[1], D. OSullivan[1]. [1]National Institute of Agricultural Botany, [2]The University of Cambridge, [3]The University of Bristol

PS04-184. **Identification of flagellar mastigoneme proteins from *Phytophthora*.** L. M. Blackman[1], W. Hee[1], M. Arikawa[3], S. Yamada[2], T. Suzaki[2], A. R. Hardham[1]. [1]Research School of Biology, Australian National University, Canberra, ACT, [2]Department of Biology, Graduate School of Sciences, Kobe University, Nada-ku, Kobe, Japan, [3]Department of Cardiovascular Control, Kochi Medical School, Nankoku, Kochi, Japan

PS04-185. ***In vivo* expression system for effector validation in hexaploid wheat (*Triticum aestivum* L.) using protoplast electroporation.** V. Segovia[1], C. Uauy[1,2]. [1]John Innes Centre, [2]National Institute of Agricultural Botany, Cambridge CB3 0LE, UK

PS04-186. **Development of gel and LC-MS/MS based method for proteomics analysis of pathogen induced response in *Malus* sp.** D. Baniulis[1], P. Haimi[1], S. Sikorskaite[1], A. Kaupinis[2], M. Ger[2], M. Valius[2], G. Staniene[1], D. Gelvonauskiene[1], V. Stanys[1]. [1]Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Kaunas reg., Lithuania, [2]Proteomics Centre, Vilnius University Institute of Biochemistry, Mokslininku st. 12, Vilnius LT-08662, Lithuania

PS04-187. **Alternative splicing of a multi-drug transporter from *Pseudoperonospora cubensis* generates an RXLR effector protein that elicits a rapid cell death.** E. A. Savory[1], C. Zou[1], B. N. Adhikari[1], J. P. Hamilton[1], C. R. Buell[1], S. Shiu[1], B. Day[1]. [1]Michigan State University

PS04-188. **Characterization of regulated protein secretion in *Phytophthora* zoospores.** W. Zhang[1], L. M. Blackman[1], A. R. Hardham[1]. [1]Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, Australia

PS04-189. **Characterisation of gene families encoding cell wall degrading enzymes in *Phytophthora*.** A. R. Hardham[1], D. Cullerne[2], P. Torrena[1], L. M. Blackman[1], J. Taylor[2]. [1]Plant Science Division, Research School of Biology, CMBE, Australian National University, Canberra, ACT 2601, Australia, [2]CSIRO Plant Industry Computational Biology, Division of Plant Industry, CSIRO, Australia, 2601

PS04-190. **Genetic analysis of the incompatibility between *Lolium* isolates of *Magnaporthe oryzae* and wheat.** T. T. P. Vy[1], Y. Inoue[1], G.-S. Hyon[1], Y. Tosa[1]. [1]Graduate School of Agricultural Science, Kobe University

PS04-191. **Appressorium-localized NADPH oxidase B is essential for aggressiveness and pathogenicity in host specific toxin producing fungus *Alternaria alternata* Japanese pear pathotype.** Y. Morita[1], G.-S. Hyon[2], N. Hosogi[1], K. Morikawa[3], M. Kusaka[3], H. Nakayashiki[2], N. Inada[4], T. Tsuge[5], P. Park[1], K. Ikeda[1]. [1]Laboratory of Stress Cytology, Graduate School of Agricultural Science, Kobe University, [2]Laboratory of Plant Pathology, Graduate School of Agricultural Science, Kobe University, [3]Faculty of Agriculture, Kobe University, [4]Plant Global Education Project, Department of Biological Sciences, Nara Institute of Science and Technology, [5]Graduate School of Bioagricultural Sciences, Nagoya University

PS04-192. **The role of Nox complex components during pathogenicity of *C. purpurea*.** J. Schuermann[1], D. Buttermann[1], P. Tudzynski[1]. [1]Institute of Biology and Biotechnology of Plants, Westfaelische Wilhelms-Universitaet Muenster, Muenster, Germany

PS04-193. **Fumonisin B1 alters mitochondrial function and actin cytoskeleton during cell death induction in Arabidopsis.** N. Yao[1], C. Fang[1], J. Li[1], X. Xi[1], F. Bi[1]. [1]State Key Laboratory of

Biocontrol, Guangdong Key Laboratory of Plant Resource, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

PS04-194. **Oxidative stress and amino acid balance are essential for the interaction of the plant-pathogen *Verticillium longisporum* and its host *Brassica napus*.** S. A. Braus-Stromeyer[1], C. Timpner[1], V.-T. Tran[1], C. E. Hoppenau[1], S. Singh[1], A. Kuehn[1], H. Kusch[1], O. Valerius[1], G. H. Braus[1]. [1]Molecular Microbiology and Genetics, University of Goettingen, Goettingen, Germany

PS04-195. **SWEET sugar transporters identified with the help of FRET sensors are highjacked for nutrition of pathogens.** L.-Q. Chen[1], B.-H. Hou[1], M. L. Hartung[1], X.-Q. Qu[1,5], S. Lalonde[1], J.-G. Kim[2], W. Underwood[4], G. Antony[3], F. F. White[3], S. C. Somerville[4], M. B. Mudgett[2], W. B. Frommer[1]. [1]Department of Plant Biology, Carnegie Institution for Science, Stanford, California, USA, [2]Department of Biology, Stanford University, 228A Gilbert Bioscience Building, 371 Serra Mall, Stanford, California 94305, USA, [3]Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA, [4]Energy Bioscience Institute, 130 Calvin Hall,MC5230, Berkeley, California 94720, USA, [5]2Key Laboratory of Plant and Soil Interactions, College of Resources and Environmental Sciences, China Agricultural University, 100193 Beijing, China

PS04-196. ***Magnaporthe oryzae* evades MAMP-triggered immunity of the host plants with surface-accumulated α-1,3-glucan on the cell wall.** M. Nishimura[1], T. Fujikawa[1], A. Sakaguchi[1], Y. Nishizawa[1], E. Minami[1], S. Yano[2]. [1]National institute of Agrobiological Sciences, [2]Ritsumeikan University

PS04-197. **Enhancement of chitin elicitor responses by engineering the chitin elicitor receptor CEBiP improves disease resistance against rice blast fungus.** Y. Kouzai[1], K. Kishimoto[2], H. Kaku[3], N. Shibuya[3], E. Minami[1], Y. Nishizawa[1]. [1]Genetically Modified Organism Research Center, National Institute of Agrobiological Sciences, Ibaraki, Japan, [2]National Institute of Floricultural Science, National Agriculture and Food Research Organization, Ibaraki, Japan, [3]Department of Life Sciences, Faculty of Agriculture, Meiji University, Kanagawa, Japan

PS04-198. **Molecular and genetic approaches to explore the melon-Fusarium interaction.** M. Normantovich[1], R. Herman[1], Z. Zvirin[1], N. Stobvun[1], O. Yogev[1], T. Goldenberg[1], Y. Brotman[1], I. Kovalski[1], R. Perl-Treves[1]. [1]Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel

PS04-199. **Identification and functional characterization of *Phytophthora infestans* RXLR effectors suppressing flg22-triggered early signalling in both *Arabidopsis* and Tomato.** X. Zheng[1], M. Fraiture[1], L. Xiaoyu[1], H. McLellan[2], M. Armstrong[2], E. M. Gilroy[3], Y. Chen[1], P. R. J. Birch[2,3], F. Brunner[1]. [1]Department of Biochemistry, Centre for Plant Molecular Biology, Eberhard Karls University, Tuebingen, Germany, [2]Division of Plant Sciences, University of Dundee (at James Hutton Institute), Errol Rd, Invergowrie, Dundee DD2 5DA, UK, [3]Cell and Molecular Sciences, The James Hutton Institute, University of Dundee , Errol Rd, Invergowrie, Dundee DD2 5DA, UK

PS04-200. **The TritNONHOST consortium: Integrative genomic and genetic analysis of nonhost resistance across Triticeae species.** F. L. Stefanato[1], R. Delventhal[2], R. E. Niks[4], J. Rajaraman[3], S. Rehman[4], U. Schaffrath[2], P. Schweizer[3], L. Boyd[1]. [1]John Innes Centre, [2]University of Aachen, Germany, [3]IPK, Gatersleben, Germany, [4]Wageningen University, Wageningen, The Netherlands

PS04-201. **How does vesicle-mediated exocytosis contribute to fungal defense in *Arabidopsis thaliana*?** H. Kim[1], S. Haigis[1], M. M. Yoshikawa[1], C. Kwon[2], M. A. Botella[3], P. Schulze-Lefert[1]. [1]Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Deptartment of Molecular Biology, Dankook University, Yongin,Korea(South), [3]Instituto de Hortofruticultura Subtropical y Mediterranea, Universidad de Malaga,Malaga,Spain

PS04-202. **Identification of an *Arabidopsis thaliana* mutant susceptible to *Botrytis cinerea* infection.** H. Yang[1], J. Wu[2]. [1]College of Life Sciences and Technology, Kunming University, Kunming, China, [2]College of Basic Medical Sciences, Kunming Medical University, Kunming, China

PS04-203. **Unraveling plant regulatory networks by studying a NAC transcription factor's role towards biotic and abiotic stress.** Y.-J. Chen[1], D. B. Collinge[1], M. F. Lyngkjaer[1]. [1]Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark

PS04-204. **Peroxisomal and mitochondrial β-oxidation contributes to virulence in *Ustilago maydis*.** M. Kretschmer[1], J. Klose[1], J. Kronstad[1]. [1]MSL, UBC, Vancouver, Canada

PS04-205. **Two secretory proteins are regulated by ProA in *Epichloë festucae*, a mutualistic symbiont of perennial ryegrass.** A. Tanaka[1], S. Saikia[2], G. Cartwright[2], D. Takemoto[1],

M. Kato[3], T. Tsuge[1], S. Hata[1], B. Scott[2]. [1]Graduate School of BioAgricultural Sciences, Nagoya University, Nagoya, Japan, [2]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand, [3]Faculty of Agriculture, Meijo University, Nagoya, Japan

PS04-206. **Functional genomic approaches to study nonhost resistance of *Medicago truncatula* against Asian soybean rust pathogen, *Phakopsora pachyrhizi*.** Y. Ishiga[1], S. R. Uppalapati[1], S. Mittal[1], V. Doraiswamy[1], M. Bedair[1], J. Chen[1], J. Nakashima[1], R. Chen[1], H. Schultheiss[2], K. S. Mysore[1]. [1]The Samuel Roberts Noble Foundation, [2]BASF Plant Science

PS04-207. **Inheritance of *Phytophthora infestans* effector-induced hypersensitive cell death in hot pepper.** S.-Y. Kim[1], H.-A. Lee[1], S.-I. Yeom[1], S.-B. Kim[1], M.-S. Kim[1]. [1]Department of Plant Science, Plant Genomics and Breeding Institute, Seoul National Universiry, Seoul, Korea

PS04-208. **NbPDR1, a PDR-type ABC transporter, confers pre- and post-invasion resistances of *Nicotiana benthamiana* against potato late blight pathogen, *Phytophthora infestans*.** Y. Shibata[1], M. Ojika[1], K. Kawakita[1], D. Takemoto[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan

PS04-209. **Arabidopsis mutants displaying aberrant localization of the PEN3 ABC transporter have altered responses to powdery mildew fungi.** W. Underwood[1], S. Somerville[1]. [1]Energy Biosciences Institute, University of California, Berkeley, Berkeley, CA USA

PS04-210. **Screening for candidates of *PWT4*, a gene for avirulence of an *Avena* isolate of *Magnaporthe oryzae* on wheat, using whole-genome sequencing.** Y. Inoue[1], K. Yoshida[2], C. Mitsuoka[2], H. Asano[1], R. Terauchi[2], Y. Tosa[1]. [1]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]Iwate Biotechnology Research Center, Kitakami, Japan

PS04-211. **Involvement of S-nitrosylated StRanBP1 in plant defense response.** H. Kato[1], D. Takemoto[1], K. Kawakita[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

PS04-212. **Evolutionary and working models for the coupled genes of the *Pik-h* encoding blast resistance of rice.** Q. Pan[1], C. Zhai[1], L. Hua[1], N. Yao[2], F. Lin[1], Y. Zhang[1], Z. Liu[2], Z. Dong[1], L. Wang[1], L. Wang[1]. [1]South China Agricultural University, [2]Sun Yat-sen University

PS04-213. **Sequencing and analysis of the Pi50(t), a novel broad-spectrum resistance genes in rice.** J. Su[1], J. Han[1], S. Chen[1], L. Zeng[1], X. Zhu[1]. [1]Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China

PS04-214. **Identification of a novel Fusarium wilt-resistance protein from tomato.** A.-M. Catanzariti[1], G. T. Lim[1], D. A. Jones[1]. [1]Division of Plant Sciences, Research School of Biology, The Australian National University, Canberra, Australia

PS04-215. ***PEN* and jasmonic acid mediate resistance in *Arabidopsis* against *Alternaria alternata* infection.** M. Egusa[1], T. Miwa[1], H. Kaminaka[1], A. Ishikawa[2], Y. Takano[3], M. Kodama[4]. [1]Laboratory of Plant Molecular Biology, Faculty of Agriculture, Tottori University, Tottori, Japan, [2]Department of Bioscience, Fukui Prefectural University, Fukui, Japan, [3]Department of Plant-Microbe Interactions, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [4]Laboratory of Plant Pathology, Faculty of Agriculture, Tottori University, Tottori, Japan

PS04-216. **A nuclear pore complex protein, Nup75, is involved in ethylene biosynthesis for phytoalexin production of *Nicotiana benthamiana* in the defense responses against *P. infestans*.** M. Ohtsu[1], Y. Shibata[1], M. Ojika[1], H. Mori[1], K. Kawakita[1], D. Takemoto[1]. [1]Graduate School of Bioagricultural Sciences,University of Nagoya,Aichi,Japan

PS04-217. **Large-scale gene disruption in *Magnaporthe oryzae* identifies MC69, a secreted protein required for infection by monocot and dicot fungal pathogens.** H. Saitoh[1], C. Mitsuoka[1], A. Hirabuchi[1], K. Ikeda[2], H. Irieda[2], K. Yoshino[2], K. Yoshida[3], J. Win[3], S. Kamoun[3], Y. Takano[2], R. Terauchi[1]. [1]Iwate Biotechnology Research Center, Iwate, Japan, [2]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [3]The Sainsbury Laboratory, John Innes Centre, Norwich, UK

PS04-218. **Biochemical analysis of a *Magnaporthe oryzae* avirulence factor, AVR-Pii.** K. Fujisaki[1], A. Ito[1], K. Yoshida[1], H. Saitoh[1], S. Kamoun[2], R. Terauchi[1]. [1]Department of genetics and genomics, Iwate Biological Research Center, [2]Sainsbury Laboratory

PS04-219. **Identification of novel non-host resistance genes in the Arabidopsis soybean rust interaction.** C. J. G. Langenbach[1], R. Campe[1], N. Tresch[2], H. Schultheiss[2], U. Conrath[1], K. Goellner[1]. [1]Institute of Plant Physiology, RWTH-Aachen University, Aachen, Germany, [2]BASF Plant Science Company GmbH, Limburgerhof, Germany

PS04-220. **Molecular cloning and analysis of a gene family encoding xylanase in *Phytophthora***

***parasitica*.** M. Lai[1], R. Liou[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

PS04-221. **Formation of highly branched hyphae by *Colletotrichum acutatum* within the fruit cuticles of *Capsicum* spp.** C.-Y. Liao[1], M.-H. Lee[1]. [1]Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan

PS04-222. **Functional analysis of an oxidative stress-regulated gene *MfAP1* from *Monilinia fructicola*.** P.-L. Yu[1], P.-Y. Chen[1], M.-H. Lee[1]. [1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

PS04-223. **Induction and regulation of highly branched penetration structure of *Colletotrichum acutatum*.** M.-Y. Chen[1], M.-H. Lee[2]. [1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, [2]1

PS04-224. **Heterochromatic marks regulate secondary metabolite biosynthesis in *Epichloe festucae* and the symbiotic interaction of this fungal endophyte with perennial ryegrass.** T. Chujo[1], B. Scott[1]. [1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand

PS04-225. **The direct protein-protein interaction results in the arms race co-evolution between *Magnaporthe oryzae AVR-Pik* and rice *Pik*.** H. Kanzaki[1], K. Yoshida[1,2], H. Saitoh[1], A. Hirabuchi[1], L. Alaux[3,4], E. Fournier[3], D. Tharreau[4], R. Terauchi[1]. [1]Iwate Biotechnology Research Center, [2]The Sainsbury Laboratory, John Innes Center, [3]UMR-BGPI, INRA, [4]UMR-BGPI, CIRAD

PS04-226. **Arabidopsis WRKY18- and WRKY40-regulated host responses in plant immunity.** R. P. Birkenbihl[1], M. Schoen[1], C. Roth[2], A. Toeller[1], I. E. Somssich[1]. [1]Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Dept. Plant Cell Biology, Georg-August University Goettigen, Germany

PS04-227. **Necrosis and ethylene-inducing peptide-like proteins of the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis*; Contradictio in Terminis?** S. Oome[1], A. Cabral[1], G. van den Ackerveken[1]. [1]Plant-Microbe Interactions, Department of Biology, Utrecht University, Utrecht, The Netherlands

PS04-228. ***Verticillium* manipulates RNA silencing to suppress host immunity.** M. van Damme[1], E. Fradin[1], U. Ellendorff[1], B. Thomma[1]. [1]Phytopathology, WUR, Wageningen, the Netherlands

PS04-229. ***COM1* encodes a novel component of the spliceosome to regulate conidium development and virulence in *Magnaporthe oryzae*.** J. Yang[1,2], J. Sun[1], L. Kong[1], D. Wang[1], Y. Zuo[1], X. Chen[1], S. Ding[3], W. Zhao[1], J.-R. Xu[3], X. Liu[2], Y.-L. Peng[1]. [1]State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Plant Pathology, China Agricultural University, Beijing 100193, China, [2]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China, [3]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

PS04-230. **AT-box as a novel *cis*-element for a bHLH protein and a JAZ protein to regulate expression of rice defense genes.** W. Zhao[1], X. Xu[1], Y. Li[1], W. Zhang[1], J. Huang[1], J. Fan[1], M. Xue[1], Z. Guo[2], Y.-L. Peng[1]. [1]State Key Laboratory of Agrobiotechnology and Department of Plant Pathology, China Agricultural University, Beijing 100193, China, [2]Department of Plant Pathology, China Agricultural University, Beijing 100193, China

PS04-231. **MoPacC acts as a transcription repressor and an activator in *Magnaporthe oryzae* via distinct processed forms.** X. Chen[1], Y. Jun[1], D. Wang[1], J. Huang[1], J. Sun[1], M. Xue[1], W. Zhao[1], Y.-L. Peng[1]. [1]State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Plant Pathology, China Agricultural University, Beijing 100193, China.

PS04-232. **Fungal small RNAs act as effectors to suppress host immune responses.** A. Weiberg[1], M. Wang[1], H. Jin[1]. [1]Department of Plant Pathology & Microbiology, Institute for Integrative Genome Biology, University of California, Riverside, CA, USA

PS04-233. **Functional analysis of Asian soybean rust resistance pathways.** K. F. Pedley[1], A. K. Pandey[2], C. Yang[2], C. Zhang[2], M. D. Kendrick[2], M. A. Graham[1], Y. Lee[2], J. H. Hill[2], S. A. Whitham[2]. [1]USDA-Agricultural Research Service, [2]Iowa State University

PS04-234. **A complex genetic system underlies the wheat powdery mildew *Pm3 - AvrPm3* interaction.** F. Parlange[1], R. Ben David[1], D. Stirnweis[1], T. Jordan[1], L. Haldemann[1], S. Oberhaensli[1], T. Wicker[1], G. Buesing[1], E. Claverie[1], B. Keller[1]. [1]Institute of Plant Biology, University of Zurich, Zurich, Switzerland

PS04-235. **Identification of genes required for Cf-dependent hypersensitive cell death.** Q.-F. Xu[1], W.-S. Cheng[1], S.-S. Li[1], W. Li[1], Z.-X. Zhang[1], Y.-P. Xu[2], X.-P. Zhou[1,3], X.-Z. Cai[1,3]. [1]Institute of Biotechnology, Zhejiang University, [2]Center of Analysis and measurement, Zhejiang University, 866 Yu Hang Tang Road, Hangzhou 310058, China, [3]Key Laboratory of Molecular

Biology of Crop Pathogens and Insects, Ministry of Agriculture, 866 Yu Hang Tang Road, Hangzhou 310058, China

PS04-236. **Innate immunity elicitors from ascomycete *Leptosphaeria maculans* induce resistance in oilseed rape.** L. Burketova[1], M. Novakova[1,2], V. Sasek[1], P. K. Dinh[2], O. Velentova[2]. [1]Institute of Experimental Botany AS CR, [2]Institute of Chemical Technology Prague

PS04-237. **Characterization of an ammonium transporter PiAMT1 from the root endophytic symbiont *Piriformospora indica*.** Y. Ding[1]. [1]Department of Organismic Interaction, Max Planck Institute for terrestrial microbiology, Marburg, Germany

PS04-238. ***ACRTS1* and *ACRTS2* genes required for biosynthesis of host-selective ACR-toxin in the rough lemon pathotype of *Alternaria alternata*.** Y. Izumi[1], K. Ohtani[1], Y. Miyamoto[1], A. Masunaka[1], T. Fukumoto[1], K. Gomi[1], Y. Tada[1], K. Ichimura[1], K. Akimitsu[1]. [1]Laboratory of Plant Pathology, Faculty of Agriculture, Kagawa University

PS04-239. **Genetic characterization of a novel inhibitor gene in *Capsicum annuum* that represses host specific disease resistance for *Phytophthora capsici*.** G. P. Reeves[1], A. L. Monroy-Barbosa[1], P. W. Bosland[1]. [1]Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, New Mexico, USA

PS04-240. **The wound induced AP2/ERF domain transcription factor WRERF50 confers resistance to necrotrophic fungi, independent of salicylate, ethylene and jasmonate signaling pathways in Arabidopsis.** C. Wang[1], J. Huang[1], R. Zhou[1], S. Liu[1]. [1]Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, China

PS04-241. **Proteomics and phosphoproteomics of *Phytophthora infestans* life stages.** S. Resjo[1], A. Ali[1], M. Lenman[1], F. Levander[2], M. Sandin[2], E. Andreasson[1]. [1]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden, [2]Department of Immunotechnology, Lund University, Lund, Sweden

PS04-242. **Effect of Methyl jasmonate on the suppression of gray mould disease and on *PAL* defense gene expression in *Botrytis cinerea* infected grapevine berries.** D. Errampalli[1], A. Sharon[2], P. H. Goodwin[3], E. A. Bordeleau[1], K. E. Schneider[1]. [1]Agriculture and Agri-Food Canada, Vineland Station, Ontario,, [2]2Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978 Israel, [3]3School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1 Canada.

PS04-243. **Loss of function of ethylene receptor ETR1 in *Arabidopsis* reduces *Fusarium oxysporum* infection.** I. S. Pantelides[1], S. E. Tjamos[2], M. Kargakis[2], S. Pappa[2], E. C. Tjamos[2], E. J. Paplomatas[2]. [1]Cyprus University of Technology, Department of Agricultural, Sciences, Biotechnology and Food Science, Lemesos, Cyprus., [2]Agricultural University of Athens, Greece

PS04-244. **Controlling Perilla rust using plant-derived essential oils.** Md. S. Ali[1], V. Bajpai[1], S.-G. Lee[2], A. Sharma[1], K.-H. Baek[1]. [1]School of Biotechnology, Yeungnam University, Gyeongsan, Korea, [2]School of Bioresource, Andong National University, Andong 760-749, Korea

PS04-245. **Progress on the cloning of *ATR2* from *Hyaloperonospora arabidopsidis*.** A. Woods-Tor[1], V. Cevik[2], D. J. Studholme[3], M. Tor[1]. [1]National Pollen and Aerobiology Research Unit, Institute of Science and the Environment, University of Worcester, Worcester, WR2 6AJ, UK., [2]School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK, [3]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK

PS04-246. ***Rpiblb2*-mediated late blight resistance requires SGT1 and salicylic acid-mediated signaling, but not RAR1 or HSP90, in *Nicotiana benthamiana*.** S.-K. Oh[1,3], S. Kamoun[4], D. Choi[1], H. Kim[2,3]. [1]Plant Genomics and Breeding Institute, Seoul National University, Seoul, Korea, [2]Green Bio-Research Center KRIBB, Deajeon, Korea, [3]Cabbage Genomics Assisted Breeding Supporting Center, Deajeon, Korea, [4]The Sainsbury Laboratory, Norwich NR4 7UH, United Kingdom

PS04-247. **The role of *VdSteA* G protein coupled pheromone receptor in virulence and biology of the vascular wilt pathogen *Verticillium dahliae*.** I. A. Stringlis[1], I. Kalaitzoglou[1], E. J. Paplomatas[1], D. I. Tsitsigiannis[1]. [1]Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Athens, Greece

PS04-248. **The Necrosis and Ethylene inducing Protein (VdNEP) gene is implicated in symptom induction by the vascular wilt fungus *Verticillium dahliae*.** A. K. Tzima[1], E. J. Paplomatas[1], D. I. Tsitsigiannis[1], S. Kang[2]. [1]Laboratory of Plant Pathology, Agricultural University of

Athens, Athens, Greece, [2]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA

Biocontrol interactions

PS05-249. **Interaction of biological control agent *Serratia plymuthica* A30 with blackleg causing biovar 3 *Dickeya* spp. *in vitro* and *in planta*.** R. Czajkowski[1,2], W. J. de Boer[1], J. A. van Veen[2,3], J. M. van der Wolf[1]. [1]Plant Research International, Wageningen University and Research Centre, [2]Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB, The Netherlands, [3]Institute of Biology Leiden, University of Leiden, Sylviusweg 72, 2333 BE, Leiden, The Netherlands

PS05-250. **Consortia of environmentally friendly microbial for control blast, bacterial leaf blight, and sheath blight diseases on rice plants.** N. R. Mubarik[1], Y. Suryadi[2], L. M. Sudirman[1]. [1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia, [2]Indonesian Research Center for Agriculture Biotechnology and Genetic Resources (BB Biogen), Cimanggu, Bogor, Indonesia

PS05-251. ***Pseudomonas fluorescens* SBW25 secretes a biosurfactant that facilitates sliding motility and plant growth promotion.** A. Al-Sohim[1,2,3], G. A. Barrett[1], J. Gallie[2], X.-X. Zhang[2], P. B. Rainey[2,3], R. W. Jackson[1]. [1]School of Biological Sciences, University of Reading, Reading, UK, [2]New Zealand Institute for Advanced Study, Massey University at Albany, New Zealand, [3]Max Planck Institute for Evolutionary Biology, Ploen, Germany

PS05-252. ***In silico* analysis of transcriptional regulatory elements related with disease resistance.** H. A. Naznin[1], Y. Yohei[1], H. Ayaka[1], H. Mitsuro[2], Y. Yoshiharu[2]. [1]The United Graduate School of Agricultural Sciences, Gifu University, Gifu, Japan, [2]Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan

PS05-253. **Genes expressed in tissue-cultured seedlings of mountain laurel (*Kalmia latifola* L.) with colonizing *Streptomyces padanus* AOK30.** A. Meguro[1,2], K. Toyoda[1], H. Ogiyama[1], S. Hasegawa[1,2], T. Nishimura[2], H. Kunoh[1,2], T. Shiraishi[1]. [1]Laboratory of Plant Pathology and Genetic Engineering, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan, [2]Institute for Biological Process Research, Akatsuka Garden Co. Ltd., Japan

PS05-254. **Induced resistance and antibiosis a dual mode of action of *Pseudozyma aphidis* against diverse phytopathogens.** M. Levy[1], K. Buxdorf[1], A. Gafni[1], I. Rahat[1]. [1]Department of Plant Pathology and Microbiology, Hebrew University of Jerusalem

PS05-255. **The biocontrol strain *Pseudomonas fluorescens* F113 is toxic towards soil amoebae.** M. Rincon[1], M. Martin[1], R. Rivilla[1], M. Sanchez-Contreras[1]. [1]Departamento de Biologia,Universidad Autonoma de Madrid, Madrid, Spain.

PS05-256. **Suppression of Fusarium wilt disease by an organic hydroponics system.** K. Fujiwara[1,2], C. Aoyama[1], M. Takano[1], M. Shinohara[2]. [1]Graduate School of Environmental Studies, Nagoya University, Nagoya, Japan, [2]National Agricultural and Food Research Organization

PS05-257. **Analysis of microbial community in organic hydroponics solution.** C. Aoyama[1], K. Fujiwara[2], M. Takano[1], M. Shinohara[2]. [1]Graduate School of Environmental Study, Nagoya University, Japan, [2]National Institute of Vegetable and Tea Science, National Agricultural Research

PS05-258. **Successful organic hydroponics by construction of microbial ecosystem in the hydroponic solution and the suppressive effect of bacterial wilt disease.** M. Shinohara[1], K. Fujiwara[1], C. Aoyama[2], M. Takano[2]. [1]National Agriculture and Food Research Organization, [2]Nagoya University

PS05-259. **Transmission of mycoviruses by attenuating programmed cell death in *Rosellinia necatrix*.** K. Ikeda[1,2], K. Inoue[1], C. Kida[1], T. Uwamori[1], S. Kanematsu[2], P. Park[1]. [1]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]National Institute of Fruit Tree Science, NARO, Japan

PS05-260. **Multiple host adhesion factors of extracellular matrix (ECM) in *Magnaporthe oryzae*-potential target for disease control-.** H. Kitagawa[1], K. Inoue[1], S. Shimoi[1], H. Kitaoka[1], P. Park[1], K. Ikeda[1]. [1]Graduate School of Agricultural Science, Kobe University, Hyogo, Japan

PS05-261. **Latest generation of biocontrol agents developed by combining agronomic performance and omics techniques.** M. Lorito[1,2], M. Ruocco[1], V. Francesco[2], S. Lanzuise[1], R. Marra[1], R. Varlese[1], F. Scala[1], S. L. Woo[1]. [1]Department ARBOPAVE-Plant Pathology, University of Naples Federico II, Italy, [2]CNR Institute for Plant Protection, Portici Naples Italy

PS05-262. ***Pseudomonas*-mediated induced systemic resistance, what is in it for the bacteria.** P. A. H. M. Bakker[1], R. F. Doornbos[1], R. L. Berendsen[1], C. M. J. Pieterse[1]. [1]Plant-Microbe Interactions, Utrecht University, Utrecht, Netherlands

PS05-263. **Insecticidal activity of *Pseudomonas taiwanensis*.** J.-R. Liu[1,2,3], W.-J. Chen[1], M.-C. Shih[3]. [1]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan, [2]Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan, [3]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, 115, Taiwan

PS05-264. **Transcriptomic analysis of systemic resistance induced by a plant growth-promoting fungus *Penicillium simplicissimum* GP17-2.** Y. Yoshioka[1], M. H. A. Naznin[1], A. Hieno[1], M. Shimizu[2], M. Hyakumachi[2], Y. Yamamoto[2]. [1]The United Graduate School of Agricultural Sciences, Gifu University, [2]Faculty of Applied Biological Sciences, Gifu University

PS05-265. **Control of rice diseases using an extract of the shrub *Chromolaena odorata* involves induced resistance.** D. K. Nguyen[1,3,4], J. Rodríguez Algaba[1], J. C. Sørensen[2], H. Sørensen[2], K. E. Andersen[2], P. T. H. Thuy[4], T. T. T. Thuy[4], D. B. Collinge[1], H. J. L. Jørgensen[1]. [1]Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark, [2]Department of Basic Sciences and Environment, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark, [3]Department of Molecular Biotechnology, Institute of Biotechnology Research and Development, Can Tho University, 3/2 Street, Ninh Kieu District, Can Tho City, Vietnam, [4]Department of Plant Protection, College of Agriculture and Applied Biology, Can Tho University, 3/2 Street, Ninh Kieu District, Can Tho City, Vietnam

PS05-266. **Obstacle of "VIROCONTROL": vacuole-mediated programmed cell death during heterogenic incompatibility in *Rosellinia necatrix*..** T. Uwamori[1,2], K. Inoue[1], C. Kida[1], H. Kitazawa[1], S. Kanematsu[2], P. Park[1], K. Ikeda[1]. [1]Graduate School of Agricultural Science, Kobe, Kobe, Japan, [2]National Institute of Fruit Tree Science, NARO, Japan

PS05-267. **Development of agricultural material for rice using the ability of rice symbiotic bacteria.** T. Isawa[1], J. Hirayama[1], S. Kanai[1], R. Ikeuchi[1], M. Noda[1], S. Shinozaki[1]. [1]Research and Development Center, Mayekawa MFG. CO., LTD

PS05-268. **Biocontrol potential of *Bacillus* sp. towards plant pathogenic bacteria from *Dickeya* spp.** S. Jafra[1], D. Krzyzanowska[1], M. Obuchowski[2], M. Potrykus[1], E. Lojkowska[1]. [1]Department of Biotechnology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdansk, Poland, [2]Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdansk, Poland

PS05-269. **Efficacy of rice stubble degrading microorganisms, fungal antagonist and N-fixing bacterium for enhancing growth and yield of organic rice.** C. Boonnadakul[1], S. Somsook[2], B. Anurugsa[3], D. Athinuwat[1]. [1]Major of Organic Farming Management, Thammasat University, Thailand 12121, [2]Department of Agricultural Technology, Thammasat University, Thailand 12121, [3]Department of Environmental Science, Thammasat University, Thailand 12121

Plant-nematode / insect interactions

PS06-270. **CLE peptide signaling in cyst nematode parasitism.** S. Chen[1], P. Lang[1], D. Chronis[2], J. Wang[3], M. G. Mitchum[3], X. Wang[1,2]. [1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA, [2]USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA, [3]Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

PS06-271. **Molecular and functional analysis of rice-nematode interactions.** G. D. Gheysen[1], T. Kyndt[1], A. Haegeman[1], K. Nahar[1], L. Bauters[1], H. Ji[1], M. Hofte[2]. [1]Dept Molecular Biotechnology, Ghent University, Ghent, Belgium, [2]Department of Crop protection, Ghent University, Ghent, Belgium

PS06-272. **Silencing of *Myzus persicae* genes by plant mediated RNAi.** A. D. Coleman[1], M. Pitino[1,2], M. E. Maffei[2], C. J. Ridout[1], S. A. Hogenhout[1]. [1]The John Innes Centre, Norwich Research Park, Norwich, UK, [2]Plant Physiology Unit, Department of Plant Biology, Innovation Centre, University of Turin, Turin, Italy

PS06-273. **Genetical genomics of nematode parasitism.** D. M. Bird[1], M. A. Djordjevic[2], D. Nielsen[1],

V. M. Williamson[3]. [1]Bioinformatics Research Center, NC State University, [2]The Australian National University, Plant Science Division, Canberra, ACT 0200, Australia,, [3]Department of Nematology, University of California, Davis, CA, 95616

PS06-274. **Functional analysis of root-knot nematode genes and host responses during Arabidopsis infection.** C. A. Gleason[1]. [1]Georg-August University, Goettingen, Germany

PS06-275. **Cowpea aphid, *Aphis craccivora* Koch. feeding behavior and plant antioxidative response in faba bean, *Vicia faba* L. cultivars.** A. Soffan[1], S. S. Alghamdi[2], A. S. Aldawood[3]. [1]Plant Protection Department, King Saud University, Kingdom of Saudi Arabia, [2]Plant Production Department, King Saud University, [3]Plant Protection Department, King Saud University

PS06-276. **A natural diterpene as an inducer for resistance to root-knot nematode (*Meloidogyne incognita*) infection in Arabidopsis and tomato.** T. Fujimoto[1], S. Seo[2], H. Abe[3], T. Mizukubo[1]. [1]Research Team for insect & pest management, National Agricultural Research Center, Tsukuba, Japan, [2]Plant-Microbe Interactions Research Unit, National Institute of Agrobiological Sciences, [3]Experimental Plant Division, RIKEN BioResource Center

PS06-277. **Unravelling the mechanisms of resistance to bluegreen aphid and pea aphid in the model legume *Medicago truncatula*.** K. Singh[1,2], L. Kamphuis[1,2], S. Guo[2,3], J. Klingler[2,4], L. Gao[2], O. Edwards[4]. [1]The UWA Institue of Agriculture, University of Western Australia, Australia, [2]CSIRO Plant Industry, Floreat, Private Bag 5 Wembley WA 6913, Australia., [3]Key Laboratory of Genetics & Biotechnology, Ministry of Education, Nanjing Forestry University, Nanjing 210037, China, [4]CSIRO Ecosystem Sciences, Floreat, Private Bag 5 Wembley WA 6913, Australia.

PS06-278. **Application of RNAi to develop plant resistance to nematode pathogens.** M. G. K. Jones[1], J. Tan[1], H. Herath[1], S. Iqbal[1], P. Nical[1], J. Fosu-Nyarko[1]. [1]Plant Biotechnology Research Group, School of Biological Sciences and Biotechnology, WA State Agricultural Biotechnology Centre, Murdoch University, Perth, Australia

PS06-279. **Unravelling the molecular events involved during the early pathogenic interaction between *Meloidogyne incognita* and *Arabidopsis thaliana*.** A. Teillet[1], K. Dybal[1], A. J. Miller[2], R. H. C. Curtis[1], B. R. Kerry[1], J. Antoniw[1], K. Hammond-Kosack[1], P. Hedden[1]. [1]Rothamsted Research, Harpenden, UK, [2]John Innes Centre, Norwich, UK

PS06-280. **Involvement of plant CLE peptide signaling in nematode infection process in tomato.** C. Ejima[1], N. T. Bui[1], S. Sawa[1]. [1]Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan

Effector proteins

PS07-281. **Functional analysis of *Xanthomonas campestris* pv. *campestris* type III effectors using transgenic plant approach.** Y.-P. Ho[1], H. Lin[1], M.-Y. Li[1], C. M. Tan[1], J.-Y. Yang[1]. [1]Institute of Biochemistry, National Chunghsing University, Taiwan

PS07-282. **Identification of infection stage-specific effector molecules of the Asian soybean rust fungus *Phakopsora pachyrhizi*.** M. Loehrer[1], C. Schlupp[1], Y. Flaskamp[1], U. Schaffrath[1]. [1]Department of Plant Physiology, RWTH Aachen University, Aachen, Germany

PS07-283. ***Pectobacterium carotovorum* uses the type III secretion machinery to suppress systemic defense in host plants.** O. Badalyan[1], Y. Nikolaichik[1]. [1]Belarusian state university, Minsk, Belarus

PS07-284. **A rice blast fungus alpha-L-arabinofuranosidase protein MoABFb is related with *Magnaporthe oryzae* infection in rice.** J. Wu[1], Y. Wang[2], S. G. Kim[2], S. T. Kim[3], K. Y. Kang[1,2]. [1]Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, South Korea, [2]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, 660-701, South Korea, [3]Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea

PS07-285. **A *Magnaporthe oryzae* secreted effector, MoCP, activates host autophagic programmed cell death.** Y. Wang[1], J. Wu[2], S. G. Kim[1], S. T. Kim[3], K. Y. Kang[1,2]. [1]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, South Korea, [2]Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, 660-701, South Korea,, [3]Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea

PS07-286. **The biotroph *Phakopsora pachyrhizi* pretends a necrotrophic pest.** R. Campe[1], M. Loehrer[1], C. Langenbach[1], G. Beckers[1], U. Schaffrath[1], U. Conrath[1], K. Goellner[1]. [1]Plant Physiology Department, RWTH Aachen, Germany

PS07-287. **Effector CoDN3 of *Colletotrichum orbiculare* suppresses NIS1-induced cell death of *Nicotiana benthamiana*.** H. Irieda[1], K. Yoshino[1], T. Okuno[1], Y. Takano[1]. [1]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PS07-288. **The structure and evolution of barley powdery mildew effector candidates.** C. Pedersen[1], J. C. Abbott[2], G. Barton[2], L. V. Bindschedler[3], R. Cramer[3], X. Lu[4], T. Maekawa[4], L. McGuffin[3], E. Ver Loren van Themaat[4], H. Thordal-Christensen[1], R. Wessling[4], R. Panstruga[5], P. D. Spanu[2]. [1]Department of Agriculture and Ecology, University of Copenhagen, Denmark, [2]Faculty of Natural Sciences, Imperial College, United Kingdom,, [3]University of Reading, United Kingdom, [4]Max-Planck Institute for Plant Breeding Research, Cologne, Germany, [5]Institute for Biology I, RWTH Aachen University, Germany

PS07-289. **Dissecting functions of *Hyaloperonospora arabidopsidis* effectors by transcriptome approach.** S. Asai[1,2], G. Rallapalli[1], G. Fabro[3], L. Wirthmueller[1], M.-C. Caillaud[1], J. Jones[1]. [1]Sainsbury Laboratory, John Innes Centre, Norwich, United Kingdom, [2]Plant Science Center, RIKEN, Kanagawa, Japan, [3]Universidad Nacional de Córdoba, Córdoba, Argentina

PS07-290. **OsPUB44, a regulator of PAMPs-induced basal resistance, is targeted by type III effector Xoo3222.** K. Ishikawa[1], K. Yamaguchi[1], K. Sakamoto[1], Y. Muraguchi[1], S. Tsuge[2], K. Shimamoto[3], C. Kojima[4], T. Kawasaki[1]. [1]Graduate School of Agriculture, Kinki University, [2]Graduate School of Agriculture, Kyoto Prefectural University, [3]Graduate School of Biological Science, Nara Institute of Science and Technology, [4]Institute for Protein Research, Osaka University

PS07-291. **Biogenesis of sRNAs homologous to effector-encoding genes and transposable elements in *P. infestans*.** R. Vetukuri[1], A. Asman[1], S. Jahan[1], C. Dixelius[1]. [1]Dept. of Plant Biology & Forest Genetics, Swedish University of Agricultural Sciences & Linnean Center for Plant Biology, Uppsala, Sweden.

PS07-292. **Functional analysis of the tumor and anthocyanin-inducing effector protein Tin2 of *Ustilago maydis*.** S. Tanaka[1], T. Brefort[1], J. Kahnt[1], R. Kahmann[1]. [1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

PS07-293. **Horizontal transfer of *holPsyAE* TTSS effector gene in *Xanthomonas campestris* strains.** M. V. Mokryakova[1], A. N. Ignatov[2,3], S. A. Bruskin[1,4]. [1]Institute of General Genetics by N.I. Vavilov, RAS, Moscow, Russia, [2]Center Bioengineering RAS, 117312, Moscow, [3]Russian Research Institute of Phytopathology RASKHN, 149050, Moscow Region, Russia, [4]Moscow Institute of Physics and Technology, 9 Institutskii pereulok, Dolgoprudny, 141700 Moscow Region, Russia

PS07-294. **Arabidopsis powdery mildew effector proteins target highly connected host proteins and display virulence activity.** R. Wessling[1], A. Stephens[1], E. Ver Loren van Themaat[1], P. Braun[2], J. L. Dangl[3], R. Panstruga[1,4]. [1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Plant Systems Biology, Center of Life and Food Sciences Weihenstephan, Technical University Munich, Munich, Germany, [3]Howard Hughes Medical Institue and Biology Department, University of North Carolina, Chapel Hill, North Carolina, United States of America, [4]Unit of Plant Molecular Cell Biology, Institute for Biology I, RWTH Aachen University, Aachen, Germany

PS07-295. ***Xanthomonas campestris* Type III effector XopJ targets the host cell proteasome to suppress plant defence.** S. Uestuen[1], V. Bartetzko[1], F. Boernke[1]. [1]Department of Biochemistry, Friedrich-Alexander University Erlangen-Nuernberg

PS07-296. **Functional characterization of small, cysteine-rich secreted effectors from the filamentous fungus *Magnaporthe oryzae*.** W. C. Sharpee[1], Y. Oh[1], B. Franck[1], J. Salisbery[1], R. Dean[1]. [1]NC State University

PS07-297. ***Xanthomonas* T3S effector XopX suppresses effector triggered immunity to promote pathogenesis.** W. Stork[1], M. Soriano[1], J.-G. Kim[1], M. B. Mudgett[1]. [1]Biology Department, Stanford University, Stanford, CA

PS07-298. **Screening of *Ralstonia solanacearum* effectors suppressing host immune responses.** Y. Taguchi[1], H. Yoshioka[2], K. Akimitsu[1], K. Ichimura[1]. [1]Kagawa University, Kagawa, Japan, [2]Nagoya University

PS07-299. **Manipulation of plant immunity signalling by the Late Blight RXLR-WY effector PexRD2.** S. R. F. King[1], M. Armstrong[2], H. McLellan[2], P. Birch[2], S. Kamoun[3], M. Banfield[1]. [1]Department of Biological Chemistry, John Innes Centre, Norwich Research Park, NR4 7UH, United Kingdom, [2]Plant Pathology, The James Hutton Institute, Invergowrie, Dundee, DD2

5DA, United Kingdom, [3]The Sainsbury Laboratory, Norwich Research Park, NR4 7UH, United Kingdom

PS07-300. **Effector Avr-Pita may form complex with Pi-ta and COX11 in the mitochondria and modulate ROS production.** L. Chen[1], F. Wang[1], X. Wang[1], Y. Chen[1], X. Zhao[1], Y. Liu[1], K. Shimamoto[2]. [1]South China Agricultural University, Guangzhou, China, [2]Plant Molecular Genetics, NAIST, 8916-5 Takayama, Ikoma 630-0101, Japan

PS07-301. **Families of candidate effector proteins identified from the haustorial transcriptomes of *Uromyces appendiculatus* and *Phakopsora pachyrhizi*.** T. I. Link[1]. [1]Department of Phytomedicine, University of Hohenheim, Germany

PS07-302. **Analysis of defense-associated MIN7 protein complex in Arabidopsis.** K. Nomura[1], H. Tanaka[2], L. Imboden[1], S. He[1]. [1]Plant Research Laboratory, Michigan State University, USA, [2]Laboratory of Plant Growth and Development, Department of Biological Sciences, Graduate School of Science, Osaka University, Japan

PS07-303. **New races with unique mutations in avirulence genes overcoming tomato *Cf* resistance genes in a Japanese population of *Cladosporium fulvum*.** Y. Iida[1,2], P. van 't Hof[2], H. Beenen[2], I. Stergiopoulos[2], R. Mehrabi[2,3], A. Notsu[4], M. Kubota[1], A. Bahkali[5], K. Abd-Elsalam[5,6], F. Terami[1], J. Collemare[2], P. J. G. M. de Wit[2,5,7]. [1]National Institute of Vegetable and Tea Science, [2]Wageningen University, [3]Seed and Plant Improvement Institute, [4]Hokkaido Research Organization, [5]King Saud University, [6]Plant Pathology Research Institute, [7]Centre for Biosystems Genomics

PS07-304. **Functional studies of *Pseudomonas syringae* type III effector AvrE.** X. Xin[1], K. Nomura[1], F. Uribe[1], X. Chen[1]. [1]Department of Plant Biology/DOE-Plant Research Laboratory, Michigan State University, US

PS07-305. **Identification of effectors from *Blumeria graminis* by *Xanthomonas* type three secretion and virus-induced gene silencing based screens.** S. Qi[1], E. Whigham[1], C. P. Prieto[2], P. D. Spanu[2], R. P. Wise[1,2], A. J. Bogdanove[1]. [1]Plant Pathology Department, Iowa State University, Ames, Iowa,US, [2]Molecular Plant Pathology Department,Imperial College London

PS07-306. **Characterization of the *CoPRF1* mutant of *Colletotrichum orbiculare* defective in establishment of host infection.** K. Tanaka[1], Y. Kubo[1]. [1]Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto prefectural University, Kyoto, Japan

PS07-307. **Functional characterization of secreted effector proteins from the hemibiotrophic fungal pathogen *Colletotrichum higginsianum*.** H. Takahara[1], J. Kleemann[2], S. Hacquard[2], R. O'Connell[2]. [1]Ishikawa Prefectural University, Ishikawa, Japan, [2]Max Planck Institute for Plant Breeding Research

PS07-308. **Identification of novel bacterial effector protein involved in hypersensitive response (HR) cell death in rice.** M. Kondo[1], C. Miyata[2], H. Sasaki[2], R. Aoi[2], F.-S. Che[1,2]. [1]Bio-Science, Nagahama Institute of Bio-Science and Technology, Shiga, Japan, [2]Graduate School of Bioscience, Nagahama Institute of Bio-Science and Technology

PS07-309. **A homologue of an avirulence gene in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* race 1 functions as a virulence gene in the cabbage yellows fungus *F. oxysporum* f. sp. *conglutinans*.** T. Kashiwa[1], K. Inami[2,4], M. Fujinaga[3], H. Ogiso[3], T. Teraoka[2], T. Arie[2]. [1]United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology (TUAT), [2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT), [3]Nagano Vegetable and Ornamental Crop Experimental Station, [4]Present Address NR R&D Department, Central Research Bridgestone Corporation

PS07-310. **Elucidation of activation mechanisms of R protein Pit by the effector protein Avr-Pit.** A. Tsujimoto[1], K. Yoshida[2], R. Terauchi[2], Y. Kawano[1], K. Shimamoto[1]. [1]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology,Nara,Japan, [2]Iwate Biotechnology Research Center

PS07-311. **Phosphatidylinositol monophosphate-binding ability of *Phytophthora infestans* RXLR effector AVR3a is required for the virulence function.** T. Yaeno[1], H. Li[2], A. Chaparro-Garcia[3], S. Schornack[3], S. Koshiba[2], S. Watanabe[2], T. Kigawa[2], S. Kamoun[3], K. Shirasu[1]. [1]Plant Science Center, RIKEN, Japan, [2]SSBC, RIKEN, Japan, [3]The Sainsbury Laboratory, UK

PS07-312. **OsBPC1 targeted by Xoo1488 effector regulates chitin induced immunity in rice.** K. Yamaguchi[1], I. Masutani[1], K. Ishikawa[1], T. Kawasaki[1]. [1]Department of Advanced Bioscience, Faculty of Agriculture, Kinki University, Nara, Japan

PS07-313. ***Magnaporthe oryzae* AVR-Pia protein: induction of resistance reaction in *Pia* rice and preparation of anti-AVR-Pia antibody.** Y. Sato[1], T. Ose[2], R. Terauchi[3], T. Sone[1]. [1]Graduate school of Agriculture, Hokkaido University, Sapporo, Japan;, [2]Research faculty of

Pharmacology, Hokkaido Univ., Sapporo, Hokkaido, Japan, [3]Iwate Biotechnology Research Center, Kitakami, Iwate, Japan.

PS07-314. **New clues to the functions of AWR effectors from *Ralstonia solanacearum* by heterologous expression in yeast.** C. M. Popa[1], M. Sole[1], J. Arino[2], M. Valls[1]. [1]Genetics Department, University of Barcelona, Barcelona, Spain, [2]Biochemistry and Molecular Biology Department & Biotechnology and Biomedicine Institute, Autonomous University of Barcelona, Barcelona, Spain

PS07-315. **Using heterologous expression approaches to study the biological functions of *Xanthomonas campestris* pv. *campestris* type III effectors (T3Es).** W.-L. Deng[1], J.-Y. Tzeng[1], J.-J. Wu[1]. [1]The Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

PS07-316. **Effectoromics of the phytopathogenic nematode, *Globodera rostochiensis*.** S. Ali[1,2], M. Magne[1], O. Côté[1], B. Mimee[2], G. Bélair[2], P. Moffett[1]. [1]Département de Biologie, Université de Sherbrooke, [2]Horticulture R & D Centre Agriculture and Agri-Food Canada 430, Boulevard Gouin, St-Jean-sur-Richelieu, Québec Canada

PS07-317. **Effector protein trafficking from *Piriformospora indica* and their function in barley root cells.** M. Rafiqi[1], F. Zhang[1], D. Biedenkopf[1], K.-H. Kogel[1]. [1]Institute of Phytopathology and Applied Zoology, Research Centre for BioSystems, LandUse, and Nutrition (IFZ), Justus Liebig University, Giessen, Germany

PS07-318. **The *Pseudomonas syringae* HopA1 effector is differentially recognized by plants and resembles phosphothreonine lyases from animal pathogens.** T. Y. Toruno[1], A. Singer[2], M. Guo[1], A. Savchenko[2], J. R. Alfano[1]. [1]Center for Plant Science Innovation and Department of Plant Pathology, University of Nebraska-Lincoln, Nebraska, USA, [2]C.H. Best Institute, University of Toronto, Toronto, Ontario, Canada

PS07-319. **Characterization of *CbAve1* from *Cercospora beticola*.** M. D. Bolton[1]. [1]USDA - ARS

PS07-320. **Transcriptome profiling identifies a novel *Xanthomonas* TALE-specific plant resistance gene in pepper.** T. Strauss[1], R. van Poecke[2], A. Strauss[1], J. Elsaesser[1], I. Ramirez[1], M. Prins[2], T. Lahaye[1]. [1]Institute of Genetics, Faculty of Biology, Ludwig-Maximilans-University Munich, Munich, Germany, [2]Keygene, Wageningen, Netherlands

PS07-321. **Development of novel fluorescent tags to monitor bacterial effector delivery *in vivo*.** J. Mathieu[1], S. Schwizer[1], G. B. Martin[1]. [1]Boyce Thompson Institute, Ithaca, NY, USA

PS07-322. **Computational and molecular identification of *Xanthomonas oryzae* TAL effector targets in rice.** A. Cernadas[1], E. Doyle[1], L. Wang[1], A. J. Bogdanove[1]. [1]Iowa State University

PS07-323. **Three new pathogenicity effectors of Pierces disease in *Xylella fastidiosa* not found in biocontrol strain EB92-1.** S. Zhang[1], P. Chakrabarty[2], D. Hopkins[3], D. W. Gabriel[1]. [1]Plant Pathology Dept., University of Florida, Gainesville, FL , USA, [2]Central Institute for Cotton Research, Nagpur, India, [3]Mid-Florida REC, University of Florida, Apopka, Florida, USA

PS07-324. **GRP7, a substrate of *Pseudomonas syringae* type III effector HopU1, plays a role in plant innate immunity by binding to immunity-related RNA.** A. Joe[1], B. Jeong[2], V. Nicaise[3], C. Korneli[4], D. Staiger[4], C. Zipfel[3], J. R. Alfano[2]. [1]School of Biological Science and Center for Plant Science Innovation, University of Nebraska, Lincoln, Nebraska 68588, USA, [2]Center for Plant Science Innovation and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68588, USA, [3]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK, [4]Molecular Cell Physiology, University of Bielefeld, 33501 Bielefeld, Germany

PS07-325. **The *Pseudomonas syringae* type III effectors HopK1 and AvrRps4 are processed during import into chloroplasts.** G. Li[1], J. R. Alfano[1]. [1]Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska, USA

PS07-326. ***Xanthomonas* Type III effector XopD desumoylates tomato transcription factor SlERF4 to suppress ethylene responses and promote pathogen growth.** J.-G. Kim[1], W. F. J. Stork[1], M. B. Mudgett[1]. [1]Dept. of Biology, Stanford University, Stanford, USA

PS07-327. **Dissecting the interaction between *P. syringae* pv. *phaseolicola* and its non-host *A. thaliana* using effectoromics.** T. Wroblewski[1], N. Belter[1], R. W. Michelmore[1]. [1]The Genome Center, University of California, Davis, Davis , CA, USA

PS07-328. **Identification and characterization of intracellular effectors Crinklers of the Oomycete *Aphanomyces euteiches*, a root pathogen of legumes.** D. Ramirez-Garces[1], Y. Martinez[1], B. Dumas[1], E. Gaulin[1]. [1]Laboratoire de Recherche en Sciences Végétales (LRSV), UM5546 CNRS-Univ Toulouse III, Pôle de Biotechnologie Végétale, Castanet-Tolosan, France.

Plant-virus / viroid interactions

PS08-329. **Seeing the world outside: a virus uses the host sensorial system to take cues from the environment.** A. Bak[1], A. Martiniere[1], J.-L. Macia[1], D. Gargani[1], S. Blanc[1], M. Drucker[1]. [1]Institut National pour la Recherche Agronomique, Montpellier, France

PS08-330. **Tomato SlSnRK1 protein interacts with and phosphorylates βC1, a pathogenesis protein encoded by a geminivirus betasatellite.** Q. Shen[1], Z. Liu[1], F. Song[1], Q. Xie[2], L. Hanley-Bowdoin[3], X. Zhou[1]. [1]Insitute of Biotechnology, Zhejiang University, Hangzhou, China, [2]State Key Laboratory of Plant Genomics, National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, [3]Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, North Carolina,USA

PS08-331. **Molecular characterization of *Chilli leaf curl virus* and satellite DNA associated with tomato in Oman.** A. J. Khan[1], A. M. Al-Zaidi[1], M. S. Akhtar[1]. [1]Sultan Qaboos University, [2]Sultan Qaboos University, [3]Sultan Qaboos University, [4]Sultan Qaboos University

PS08-332. **Functional analysis of *Cucumber mosaic virus* 2b protein and coat protein on symptom development of inoculated tobacco plant.** T. Mochizuki[1], T. Wada[1], Y. Hirata[1], S. T. Ohki[1]. [1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan.

PS08-333. **5' untranslated region of tobamovirus RNA is involved in viral cell-to-cell movement.** H. Mizumoto[1], A. Kiba[1], Y. Hikichi[1]. [1]Laboratory of Plant Pathology and Biotechnology, Kochi University, Kochi, Japan

PS08-334. **Characterization of a ribonucleoprotein complex that serves as a precursor of tobacco mosaic virus replication complex.** K. Kawamura[1], K. Ishibashi[1], M. Ishikawa[1]. [1]Plant-Microbe Interactions Research Unit, National Institute of Agrobiological Sciences, Tsukuba, Japan

PS08-335. **Next generation sequencing reveals chrysanthemum genes and small RNAs associated with *Chrysanthemum stunt viroid*.** Y. Jo[1], K.-M. Jo[1], K.-H. Kim[1], W. K. Cho[1]. [1]Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, 151-921, Republic of Korea

PS08-336. **A putative sodium-hydrogen antiporter helps *Bamboo mosaic virus* accumulation in *Nicotiana benthamiana*.** M. Meng[1], Y.-T. Han[1], H.-C. Wu[1], T.-Y. Ou[1]. [1]National Chung Hsing University

PS08-337. **Do non-circulative plant viruses sense the arrival of the aphid vector?** M. Drucker[1], A. Bak[1], J.-L. Macia[1], S. Blanc[1]. [1]INRA, UMR BGPI Plant Pathogen Interactions, Montpellier, France

PS08-338. **The interaction proteome of the N NB-LRR immune receptor.** P. Cournoyer[1], J. L. Caplan[2], B. S. Phinney[3], S. P. Dinesh-Kumar[3]. [1]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, USA, [2]Department of Biological Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA, [3]Department of Plant Biology and The Genome Center, College of Biological Sciences, University of California, Davis, CA, USA.

PS08-339. **Management of whitefly transmitted begomovirus associated with tomato in Oman.** A. A. Al-Shihi[1], A. J. Khan[1]. [1]Sultan Qaboos University

PS08-340. **Regulation of the cell-to-cell movement of plant viruses by a Ser/Thr kinase-like protein.** C.-P. Cheng[1], S.-F. Cheng[2], M.-S. Tsai[1], C.-H. Tsai[2]. [1]Department of Life Sciences, Tzu Chi Univeristy, Hualien, Taiwan, [2]Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan

PS08-341. **Mutations in the 130K/180K replication protein genes of *Pepper mild mottle virus* that confer the ability to systemically infect tomato plants reduce its infectivity in original hosts.** Y. Morikawa[1], K. Ishibashi[2], H. Mizumoto[1], K. Kimura[1], K. Matsumoto[1], A. Kiba[1], M. Ishikawa[2], T. Okuno[3], Y. Hikichi[1]. [1]Laboratory of Plant Pathology and Biotechnology, Kochi University, Kochi, Japan, [2]Division of Plant Sciences, National Institute of Agrobiological Sciences, Tsukuba, Japan, [3]Laboratory of Plant pathology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PS08-342. **Recapitulation of ribosomal frameshifting of *Clover yellow vein virus* P3N-PIPO in a cell-free translation system.** Y. Hagiwara-Komoda[1], S. H. Choi[1], K. Nakahara[1], S. Naito[1]. [1]Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

PS08-343. **Multiple suppressors of posttranscriptional gene silencing encoded by *Ageratum yellow vein virus*, a monopartite begomovirus.** M. S. Shahid[1], M. Ikegami[2], P. Sharma[3], T. Kon[4], K. T. Natsuaki[1], M. S. Shahid[1,5]. [1]Department of International Agricultural Development, Tokyo University of Agriculture, Tokyo 156-8502, Japan, [2]NODAI Research Institute, Tokyo University of Agriculture, Tokyo 156-8502, Japan, [3]Division of Crop Improvement, Directorate of Wheat Research, Karnal 132001, India, [4]Department of Plant Pathology University of California, Davis, USA, [5]Department of Biosciences, COMSATS Institute of Information Technology, Sahiwal 57000, Pakistan

PS08-344. **Toward molecular isolation of the *Pvr4* gene conferring resistance against *Pepper mottle virus* in *Capsicum annuum*.** S.-B. Kim[1], J.-H. Han[2], H. J. Kim[1], S.-Y. Kim[1], D. Choi[1]. [1]Department of Plant Science, Seoul National University, Seoul, Korea, [2]Pepper and Breeding Institute, Business Incubator, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-853, Korea

PS08-345. **Screening for virulence factors of *Gentian Kobu-sho-associated virus* involved in tumorous or hyperplastic disorders in gentian.** G. Atsumi[1], R. Tomita[1], K. Kobayashi[2], H. Saitoh[1], K.-T. Sekine[1]. [1]Iwate Biotechnology Research Center, [2]Ehime University

PS08-346. **Evaluation of the durability of *N'* resistance gene to *Pepper mild mottle virus* using random mutagenesis of coat protein genes.** K. Idehara[1], M. Noguchi[1], R. Tomita[2], G. Atsumi[2], K.-T. Sekine[2], N. Yamaoka[3], M. Nishiguchi[1], K. Kobayashi[1]. [1]Laboratory of Plant Molecular Biology and Virology, Faculty of Agriculture, Ehime University, Ehime, Japan, [2]Research group of Plant Pathology, Iwate Biotechnology Research Center, Iwate, Japan, [3]Laboratory of Plant Pathology, Faculty of Agriculture, Ehime University, Ehime, Japan

PS08-347. **Increased expression of P3N-PIPO facilitate the cell-to-cell movement of *Clover yellow vein virus* in a *cyv1*-resistant pea.** S. H. Choi[1], K. S. Nakahara[1], I. Uyeda[1]. [1]Pathogen-plant Interactions group, The Graduate School of Agriculture, Hokkaiod Universuty, Sapporo, Japan

PS08-348. **A thioredoxin h protein from *Nicotiana benthamiana* is involved in the movement of *Bamboo mosaic virus*.** Y.-P. Huang[1], H.-T. Chen[1], L.-L. Shenkwen[1], S.-F. Cheng[1], Y.-H. Hsu[1], C.-H. Tsai[1]. [1]Graduate Institute of Biotechnology, University of Chung Hsing,Taichung,Taiwan

PS08-349. **Determining the mechanism by which the p8 and p6.6 proteins from *Panicum mosaic virus* influence its intercellular movement in maize.** X. Ding[2], E. B. Blancaflor[1], M. Zhu[2], R. S. Nelson[1]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, OK, USA, [2]Plant Pathology Department, China Agricultural University, Beijing, China

PS08-350. **Evidence that SGT1 facilitates viral accumulation and induction of necrosis in *Tomato ringspot virus* infected plants.** B. Ghoshal[1], H. Sanfacon[2]. [1]Department of Botany, The University of British Columbia, Vancouver, Canada, [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland,BC, Canada

PS08-351. ***Cucumber mosaic virus* (CMV) RNA3 transgenic *Nicotiana benthamiana* complement to express CMV -RNA1 and RNA2 systemically.** N. Fukuzawa[1], T. Matsumura[1], C. Masuta[2]. [1]National Institute of Advanced Industrial Science and Technology, Sapporo, Japan, [2]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

PS08-352. **Identification of domains in p27 auxiliary replicase protein essential for its association with the endoplasmic reticulum membranes in *Red clover necrotic mosaic virus*.** K. Kusumanegara[1], A. Mine[2], K. Hyodo[1], M. Kaido[1], K. Mise[1], T. Okuno[1]. [1]Laboratoty of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany

PS08-353. **The regulation mechanism of reactive oxygen species generation by calcium-dependent protein kinase.** M. Kamimura[1], Y. Kousaka[1], Y. Han[1], F.-S. Che[1]. [1]Bio-Science, Graduate School of Bio Sciences, Nagahama Institute of Bio-Science and Technology, Shiga, Japan

PS08-354. **Effect of rice RNA-dependent RNA Polymerase 1 (OsRDR1) on RNA silencing and small RNA regulation.** M. Nishiguchi[1,2], H. Chen[1,2], K. Kobayashi[1], N. Yamaoka[1]. [1]Faculty of Agriculture, Ehime University, Matsuyama, Japan, [2]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada

PS08-355. **Maize Ferredoxin-5 plays a negative role in *Sugarcane mosaic virus* infection.** K.-S. Wang[1], Y. Cheng[1]. [1]Department of Pomology,College of Agronomy and Biotechnology, China Agricultural University

PS08-356. **Ultrastructural study of *Tomato yellow leaf curl virus* in the cells of host plants and the midgut epithelial cells of the insect vector, whitefly.** M. Uchibori[1], M. Suzuki[1], M. Ugaki[1]. [1]The Department of Integrated Biosciences, The University of Tokyo, Chiba, Japan

PS08-357. **Evaluation of RNAi-mediated resistance offered to *Potato spindle tuber viroid* in transgenic *N. benthamiana* plants expressing different hairpin RNA constructs.** C. R. Adkar-Purushothama[1], A. Kasai[1], T. Harada[1], T. Sano[1]. [1]Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Japan

PS08-358. **A seed storage protein, PAP85, involved in early stage of replication of *Tobacco mosaic virus* and ER morphology change.** C.-E. Chen[1], T.-T. Wang[1], I.-L. Chien[1], H.-H. Yeh[1,2]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan., [2]Research Center for Plant Medicine, National Taiwan University, Taipei, Taiwan.

PS08-359. **Molecular characterization of *Potato spindle tuber viroid*-derived and non-related circular RNAs from dahlia.** T. Tsushima[1], T. Sano[1]. [1]Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan

PS08-360. **Identification of the amino acids in Cap binding pocket of *Brassica rapa* eIF(iso)4E inducing the resistance against *Turnip mosaic virus*.** J. Kim[1], W. Kang[1], D. Kim[2]. [1]Department of Plant Science, CALS, Seoul National University, Seoul, [2]National Institute of Horticultural and Herbal Science, Suwon 440-706, Korea

PS08-361. **Seasonal dynamics and correlation studies of two viroids in two citrus cultivars.** C.-Y. Lin[1], T.-H. Hung[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

PS08-362. **Analysis of nucleo-cytoplasmic trafficking of the *Turnip crinkle virus* coat protein and its influence on plant defense responses.** J. Y. Moon[1,3], W. D. Heo[2], J. M. Park[1,3]. [1]Department of Biosystems and Bioengineering, University of Science and Technology, [2]Department of Biological Sciences, KAIST, [3]Green Bio Research Center, KRIBB

PS08-363. **Identification of host proteins interacting with the capsid protein of *Odotoglossum ringspot virus*.** W.-C. Hu[1], S.-C. Lee[1], C.-H. Mao[1], Y.-C. Chang[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

PS08-364. **Induction of tobamovirus resistance in nontransgenic scions after grafting onto *NtTOM1* and *NtTOM3* silenced rootstocks.** Md. E. Ali[1], K. Kobayashi[1], N. Yamaoka[1], M. Ishikawa[2], M. Nishiguchi[1]. [1]Faculty of Agriculture, Ehime University, 3-5-7 Tarumi. Matsuyama 790-8566, Japan, [2]National Institute of Agrobiological Sciences, 3-10-3 Kan-nondai, Tsukuba, Ibaraki 305-602, Japan

PS08-365. ***RCY1*-mediated resistance to *Cucumber mosaic virus* is regulated by LRR domain-mediated interaction with CMV(Y) following degradation of RCY1.** H. Takahashi[1], H. Shoji[1], S. Ando[1], M. Takeshita[2], M. Suzuki[3], C. Masuta[4]. [1]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan, [2]Faculty of Agriculture, Kyushu University, Fukuoka, Japan, [3]Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan, [4]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

PS08-366. **Assessment of RNA exosome as a viral resistance factor.** N. Kumakura[1], A. Takeda[1], Y. Watanabe[1,2]. [1]Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan, [2]Graduate Program on Environmental Sciences (GPES)

PS08-367. **The expression of miR398 and its target genes in BaMV transgenic *Nicotiana benthamiana* plants.** F.-C. Hsu[1], S.-K. Yen[1], B.-N. Shen[1], Y.-C. Lee[2], Y.-H. Hsu[2], N.-S. Lin[1]. [1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, [2]Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan

PS08-368. **Host glycine rich protein 2 has a role in plant defense to virus infection.** H.-C. Chen[1], Y.-T. Tu[1], Y.-H. Hsu[2], N.-S. Lin[1]. [1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei 115, Taiwan, [2]Graduate Institute of Biotechnology, National Chung Hsing University, Taichung 402, Taiwan

PS08-369. **Transgenic expression of TMV capsid and movement proteins modulate plant basal defense and biotic stress responses in *Nicotiana tabacum*.** G. Conti[1], M. C. Rodriguez[1], C. A. Manacorda[1], S. Asurmendi[1]. [1]Instituto de Biotecnologia CICVyA-INTA, Hurlingham, Argentina

PS08-370. **Study of the involvment of the genes that encode the proteins SlGAL83 and TCTP in the infection of a susceptible host by *Pepper yellow mosaic virus*.** R. S. Cascardo[1], F. P. Bruckner[1], A. S. Xavier[2], F. M. Zerbini[2], P. A. Zerbini[1]. [1]Department of Microbiology, University of Vicosa, Vicosa, Brazil, [2]Department of Phytopathology, University of Vicosa

PS08-371. **Viral infection dynamics and interference under the synergism between *Cucumber mosaic virus* and *Turnip mosaic virus*.** M. Takeshita[1], E. Koizumi[1], M. Noguchi[1], N. Furuya[1],

K. Tsuchiya[1]. [1]Faculty of Agriculture, Kyushu University, Fukuoka, Japan

PS08-372. **Functions of the coat protein of *Potato virus A* are regulated by protein kinase CK2 phosphorylation and by a pathway involving cellular HSP70 and its co-chaperon CPIP.** A. Lohmus[1], A. Hafren[1], K. Makinen[1]. [1]Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland

PS08-373. **Discovery and characterization of a novel calarvirus infecting potatoes in China.** Y.-Y. Li[1], R.-N. Zhang[1], H.-Y. Xiang[1], H. Abouelnasr[1], D.-W. Li[1], J.-L. Yu[1], J. H. McBeath[2], C.-G. Han[1]. [1]Department of Plant Pathology and State Key Laboratory for Agro-biotechnology, China Agricultural University,Beijing, P. R. China, [2]Plant Pathology and Biotechnology Laboratory, Agriculture and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks, USA

PS08-374. **Two distinct sites are essential for virulent infection and support of variant satellite RNA replication in spontaneous *Beet black scorch virus* variants.** J. Xu[1], X. Wang[1], L. Shi[1], Y. Zhou[1], D. Li[1], C. Han[1], Z. Zhang[1], J. Yu[1]. [1]State Key Laboratory of Agro-Biotechnology, China Agricultural University, Beijing 100193, China

Cell wall modification and resistance

PS09-375. **Extracellular apyrase (ecto-ATPase) regulates the peroxidase-catalyzed apoplastic oxidative burst in cowpea (*Vigna sinensis* Endl.): implication in nonhost resistance.** K. Tanaka[1], K. Toyoda[1], N. Yamagishi[2], N. Yoshikawa[2], Y. Inagaki[1], Y. Ichinose[1], T. Shiraishi[1]. [1]Laboratory of Plant Pathology and Genetic Engineering, Graduate school of Environmental and Life Scirence, Okayama University, Okayama, Japan, [2]Laboratory of Plant Pathology, Faculty of Agriculture, Iwate University, Japan

PS09-376. **Defense-related LsGRP1 protein may link to cell wall pectin and involve in disease resistance regulation *via* protein-protein interaction.** C.-H. Lin[1], C.-Y. Chen[1]. [1]Department of Plant Pathology & Microbiology, National Taiwan University, Taipei, Taiwan

PS09-377. **SignWALLing: Signals derived from Arabidopsis cell wall activate specific resistance to pathogens.** E. Miedes[1], M. P. Riviere[1], A. Sanchez-Vallet[1], C. Sanchez-Rodriguez[1], P. Ranocha[2], X. Bartel[3], Y. Marco[3], D. Goffner[2], A. Molina[1]. [1]Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Departamento Biotecnología. Polytechnic University of Madrid, Madrid, Spain, [2]Unite; Mixte de Recherche Centre Nacional de la Recherche Scientifique Univ Toulouse III, Pole de Biotechnologie Vegetale, BP 42617 Auzeville 24, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE, [3]Laboratoire de Interactions Plantes-Microorganismes, Centre Nacional de la Recherche Scientifique Instiut National de la Recherche Agronomique, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE.

PS09-378. **Plant response to danger signals.** T. J. Kariola[1], P. Davidsson[1], M. Piisila[1], K. Sims-Huopaniemi[1], T. Palva[1]. [1]Division of Genetics, Department of Biosciences, University of Helsinki, Finland

PS09-379. **Infection inhibitor(s) generated in the cell wall preparation from *Pisum sativum* L.** K. Iio[1], C. Kamada[1], T. Watanabe[1], M. Izumi[1], Y. Inagaki[1], Y. Ichinose[1], K. Toyoda[1], T. Shiraishi[1]. [1]Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan

PS09-380. **The apoplastic oxidative burst and induced extracellular defense: production of an anti-fungal compound(s) in the extracellular space of cowpea leaves challenged with the fungal elicitor.** M. Uchioki[1], K. Toyoda[1], K. Tanaka[1], M. Takagi[1], Y. Inagaki[1], Y. Ichinose[1], T. Shiraishi[1]. [1]Faculty of Agriculture, Okayama University, Okayama, Japan

PS09-381. **Sub-cellular dynamics of beta-1,3-glucanases during stress response.** R. Zavaliev[1], A. Levy[1], B. L. Epel[1]. [1]Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel

PS09-382. **Activities of 9-lipoxygenase in controlling plant defence and cell wall integrity.** T. Vellosillo[1], J. Vicente[1], S. Kulasekaran[1], V. Aguilera[1], M. Martinez[1], R. Marcos[1], Y. Izquierdo[1], M. Hamberg[2], C. Castresana[1]. [1]Centro Nacional de Biotecnologia, CSIC, [2]Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden

Plant hormones integrating defense response

PS10-383. **The vascular pathogen *Verticillium longisporum* exploits a jasmonic acid-independent COI1 function in roots to enhance disease symptoms in *Arabidopsis thaliana* shoots.** C. Thurow[1], A. Ralhan[1], S. Schoettle[1], T. Iven[1], I. Feussner[1], A. Polle[2], C. Gatz[1]. [1]Albrecht-von-Haller Institute for Plant Sciences, Georg-August-University, Goettingen, Germany, [2]Buesgen Institute, Georg-August-University, Goettingen, Germany

PS10-384. **Compensatory functions of salicylic acid and MAPK signaling in effector-triggered immunity.** K. Tsuda[1,2], A. Mine[1], J. Glazebrook[2], F. Katagiri[2]. [1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Center for Microbial and Plant Genomics, Department of Plant Biology, University of Minnesota, St. Paul, USA

PS10-385. **The role of plant hormones in the interaction between rice roots and nematodes.** T. Kyndt[1], K. Nahar[1], G. Gheysen[1]. [1]Department of Molecular Biotechnology, Ghent University

PS10-386. ***Moniliophtora perniciosa-Solanum lycopersicum* interaction in tomato hormonal mutants.** J. Deganello[1], G. A. Leal Jr[2], L. E. P. Peres[3], A. Figueira[1]. [1]Centro de Energia Nuclear na Agricultura, Universidade de Sao Paulo, Piracicaba, SP, Brazil, [2]Universidade Federal de Alagoas, Maceio, AL, Brazil, [3]Escola Superior de Agricultura "Luiz de Queiroz", Universidade de Sao Paulo, Piracicaba, SP, Brazil

PS10-387. **Profiling of specific proteins induced in Japanese birch plantlet treated with salicylic acid or azelaic acid.** S. Yokota[1], H. Suzuki[1], F. Ishiguri[1], K. Iizuka[1], N. Yoshizawa[1]. [1]Department of Forest Science, Utsunomiya University, Utsunomiya, Japan

PS10-388. **Rice WRKY62 is a positive regulator of SA-pathway-mediated regulation of diterpenoid phytoalexin synthesis genes in rice.** S. Fukushima[1], A. Akagi[1], S. Sugano[1], S. Goto[1], H. Takatsuji[1]. [1]National Institute of Agrobiological Sciences, Tsukuba, Japan

PS10-389. **Genetic dissection of jasmonate-flagellin antagonism.** X. Zhang[1], Y. Millet[1], F. Ausubel[1]. [1]Massachusetts General Hospital/Harvard Medical School

PS10-390. **Translocation of phospholipase A2α to apoplasts is modulated by developmental stages and bacterial infection in Arabidopsis.** S. B. Ryu[1]. [1]Korea Research Institute of Bioscience & Biotechnology

PS10-391. **Proteome and transcriptome analysis of wound-induced accumulation of salicylic acid in WIPK/SIPK-suppressed plants.** S. Katou[1], Y. Onishi[2], N. Asakura[2], I. Mitsuhara[3], S. Seo[3]. [1]International Young Researchers Empowerment Center, Shinshu University, [2]Graduate School of Agriculture, Shinshu University, [3]National Institute of Agrobiological Sciences

PS10-392. **VOZ governs abiotic and biotic stress responses in *Arabidopsis*.** M. H. Sato[1], Y. Nakai[2], Y. Nakahira[1], K. Takebayashi[3], Y. Nagasawa[3], K. Yamasaki[1], M. Ohme-Takagi[3], S. Fujiwara[3], N. Mitsuda[3], E. Fukusaki[3], H. Sumida[1], Y. Kubo[1]. [1]Graduate School of Life and Environment. Sciences., Kyoto Prefectural University, Kyoto, Japan, [2]Bioproduction Research Institute, AIST, Tsukuba, 305-8562, Japan, [3]Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita, Osaka, 565-0871, Japan

PS10-393. **The nuclear ubiquitin proteasome regulates WRKY45 function in a dual mode in the rice defense program.** A. Matsushita[1], H. Inoue[1], S. Goto[1], A. Nakayama[1], S. Sugano[1], N. Hayashi[1], H. Takatsuji[1]. [1]National Institute of Agrobiological Sciences, Tsukuba, Japan

PS10-394. **Functional analysis of bHLH transcriptional factors MYL1, MYL2 and MYL 3 in jasmonate signaling.** Y. Sasaki-Sekimoto[1], H. Saito[2], S. Masuda[3], H. Ohta[3], K. Shirasu[1]. [1]Plant Immunity Research Group, RIKEN PSC, Yokohama, Japan, [2]Graduate school of Bioscience and Biotechnology, Tokyo Institute of Technology, [3]Center for Biological Resources and Informatics, Tokyo Institute of Technology

PS10-395. **MED25 integrates jasmonate associated transcription in *Arabidopsis thaliana*.** B. N. Kidd[1,2], V. Cevik[3], D. M. Cahill[4], J. M. Manners[2], K. Kazan[2], J. Beynon[3], P. M. Schenk[1]. [1]School of Agriculture and Food Sciences, The University of Queensland, St Lucia, Australia, [2]Commonwealth Scientific and Industrial Research Organization, Plant Industry, St Lucia, Australia, [3]School of Life Sciences, University of Warwick, Wellesbourne Campus, UK, [4]School of Life and Environmental Sciences, Deakin University, Geelong, Australia

PS10-396. **Function of COI1 in *N* gene-mediated resistance to *Tobacco mosaic virus* in tobacco.** K. Oka[1], M. Kobayashi[2], I. Mitsuhara[1], S. Seo[1]. [1]National Institute of Agrobiological Sciences, Ibaraki, Japan, [2]NARO Institute of Floricultural Science, Ibaraki, Japan

PS10-397. **Involvement of auxin transcriptional repressor IAA8 on the regulation of programmed cell death via direct interaction with LSD1 in *Arabidopsis*.** H. Kaminaka[1], N. Nishimoto[1], F. Arase[1], K. Takabayashi[1], K. Nishide[1], N. S. Coll[2], P. Epple[2], B. F. Holt III[3], J. L. Dangl[2].

[1]Faculty of Agriculture, Tottori University, Tottori, Japan, [2]HHMI and Department of Biology, University of North Carolina at Chapel Hill, NC 27510, USA, [3]Department of Botany and Microbiology, University of Oklahoma, OK 73019, USA

PS10-398. **Rice OsERF922 negatively regulates basal resistance and salt tolerance modulated by ABA.** D. Liu[1], X. Chen[1], J. Liu[1], Z. Guo[1]. [1]Department of Plant Pathology, China Agricultural University, Beijing, China

PS10-399. **Involvement of the JA-inductive bHLH transcription factor RERJ1 in rice defense responses.** N. Kawamura[1], K. Miyamoto[1], R. Ozawa[2], J. Takabayashi[2], A. Miyao[3], H. Hirochika[3], H. Nojiri[1], H. Yamane[4], K. Okada[1]. [1]Biotechnology Research Center, The University of Tokyo, [2]Center for Ecological Research, Kyoto University, [3]National Institute of Agrobiological Sciences, [4]Department of Biosciences, Teikyo University

PS10-400. **Jasmonate signaling pathway through JASMONATE-ZIM DOMAIN (JAZ) protein in rice.** E. Murakami[1,2], M. Yasuda[2], S. Shima[2], T. Tomizawa[1], H. Nakashita[1,2]. [1]Department of Applied Biology and Chemistry, Tokyo University of Agriculture, Tokyo, Japan, [2]Plant-Endophyte Interactions Laboratory, Center for Intellectual Property Strategies, RIKEN, Saitama, Japan

PS10-401. **Effect of environmental stress on induced disease resistance by plant activators and endophytic bacteria in rice.** M. Kusajima[1,2], J. Hirayama[2], M. Yasuda[2], S. Shinozaki[2], H. Nakashita[1,2]. [1]Department of Applied Biology and Chemistry, Tokyo University of Agriculture, Tokyo, Japan, [2]RIKEN Innovation Center, RIKEN

PS10-402. **Salicylic acid and ethylene induce resistance to *Phytophthora sojae* in soybean (*Glycine max*).** C.-J. Jiang[1], T. Sugimoto[2], S. Sugano[1]. [1]Plant Disease Resistance Research Unit, National Institute of Agrobiological Sciences, [2]Hyogo Agricultural Institute for Agriculture

PS10-403. **Development of multi-disease resistant rice by optimized overexpression of *WRKY45*.** S. Goto[1], F. Sasakura-Shimoda[1], A. Matsushita[1], H. Takatsuji[1]. [1]Disease Resistant Crops Research and Development Unit, National Institute of Agrobiological Sciences, Ibaraki, Japan

PS10-404. **Chloroplast-mediated plant innate immunity through chloroplast Ca^{2+} sensor protein CAS.** H. Nomura[1], K. Shimotani[2], K. Nakai[2], T. Furuichi[3], S. Sano[2], E. Fukusaki[4], H. Yoshioka[1], Y. Nakahira[2], T. Shiina[2]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [2]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan, [3]EcoTopia Science Institute, Nagoya University, Nagoya, Japan, [4]Graduate School of Engineering, Osaka University, Osaka, Japan

PS10-405. **Dynamic changes in histone modifications during ABA-mediated suppression of defense-related genes.** K. Fuji[1], M. Yasuda[1], H. Nakashita[1,2]. [1]Plant-Endophyte Interactions Laboratory, RIKEN Innovation Center, RIKEN, [2]Department of Applied Biology and Chemistry, Tokyo University of Agriculture

PS10-406. **Mitochondrial complex II plays a critical role in defense against diverse pathogens.** L. F. Thatcher[1], C. Gleason[1], S. Huang[2], R. C. Foley[1], A. H. Millar[2], K. B. Singh[1,3]. [1]CSIRO Plant Industry, [2]Australian Research Council (ARC) Centre of Excellence in Plant Energy Biology, University of Western Australia, Crawley, WA 6009, Australia, [3]University of Western Australia Institute of Agriculture, University of Western Australia, Crawley, WA 6009, Australia.

PS10-407. **Blast resistance by panicle blast resistant gene *Pb1* is mediated by WRKY45.** H. Inoue[1], N. Hayashi[1], A. Matsushita[1], X. Liu[1], A. Nakayama[1], S. Sugano[1], C.-J. Jiang[1], H. Takatsuji[1]. [1]National institute of agrobiological sciences

PS10-408. **Identification of naringenin 7-*O*-methyltransferase, a key enzyme in the biosynthesis of the flavonoid phytoalexin sakuranetin in rice.** T. Shimizu[1], K. Miyamoto[1], F. Lin[1], M. Hasegawa[2], H. Nojiri[1], H. Yamane[3], K. Okada[1]. [1]Biotechnology Research Center, The University of Tokyo, [2]College of Agriculture, Ibaraki University, [3]Department of Biosciences, Teikyo University

PS10-409. **The AP2/ERF domain transcription factor ERF104 confers resistance to necrotrophic fungi via salicylate and ethylene signaling pathways, but not jasmonate pathway.** C. Wang[1], J. Huang[1], C. Dong[1], S. Liu[1]. [1]Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, China

PS10-410. **Role of JAZ protein in jasmonic acid-induced resistance to rice bacterial blight in rice.** S. Yamada[1], A. Miyamoto[1], A. Kano[1], H. Shishido[1], S. Miyoshi[1], S. Taniguchi[1], D. Tamaoki[1], K.

Akimitsu[1], K. Gomi[1]. [1]Faculty of Agriculture, Kagawa University, Kagawa, Japan

PS10-411. **Role of jasmonic acid-induced volatile in resistance to rice bacterial blight in rice.** S. Taniguchi[1], Y. Hosokawa-Shinonaga[1], S. Miyoshi[1], H. Shishido[1], R. Ozawa[2], J. Takabayashi[2], D. Tamaoki[1], K. Akimitsu[1], K. Gomi[1]. [1]Faculty of Agriculture, Kagawa University, Kagawa, Japan, [2]Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113,Japan

PS10-412. **Plasmodesmata and defense signaling: mechanisms and players.** J.-Y. Lee[1], X. Wang[1], W. Cui[1], R. Sager[1], E. H. Kim[1]. [1]Delaware Biotechnology Institute, University of Delaware, Newark, DE, U. S. A.

PS10-413. **The interplay between cytokinin and salicylic acid signaling coordinates activation of defense responses in Arabidopsis.** D. Hajdu[1], D. Bush[1], J. Dangl[2], J. Kieber[2], C. Argueso[1]. [1]Biology Department, Colorado State University, [2]Department of Biology, University of North Carolina

Crop protection

PS11-414. **Characterising the genetic basis of *E. coli* O157:H7 survival in the plant environment.** G. A. Barrett[1], R. W. Jackson[1], S. C. Andrews[1], P. R. Hirsch[2], T. H. Mauchline[2], E. J. Shaw[1]. [1]School of Biological Sciences, University of Reading, Reading, UK, [2]Rothamsted Research

PS11-415. **Cloning, characterization and expression of an insecticidal crystal protein gene from *Bacilllus thuringiensis* isolates of Andaman and Nicobar Islands.** H. M. M. Swamy[1], R. Asokan[1], G. G. Thimmegowda[1], R. Mahmood[2], S. N. Nagesha[1], D. K. Arora[3]. [1]Indian Institute of Horticultural Research (IIHR), [2]Post-Graduate Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnanasahayadri, Shankaraghatta, Shimoga 577451, Karnataka, INDIA, [3]National Bureau of Agriculturally Important Micro organisms (NBAIM), Mau Nath Bhajan, Uttar Pradesh 275101.

PS11-416. **Elevation of soil microbial enzyme activities and reduction of fusarium wilt disease incidence by chitin amendment in tomato field plots.** P. Wongkaew[1], T. Homkratoke[1]. [1]Division of Plant Pathology, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

PS11-417. **Use of essential oils for the control of post harvest decay in citrus.** T. Anjum[1], N. Akhtar[1]. [1]Institute of Agricultural Sciences, University of the Punjab, Lahore-54590, Pakistan.

PS11-418. **Imprimatins, novel plant immume-priming compounds identified *via* a newly-established high-throughput chemical screening target salicylic-acid glucosyltransferases in *Arabidoposis thaliana*.** Y. Noutoshi[1], M. Okazaki[1], T. Kida[1], Y. Nishina[1], Y. Morishita[2], T. Ogawa[2], H. Suzuki[2], D. Shibata[2], Y. Jikumaru[3], A. Hanada[3], Y. Kamiya[3], K. Shirasu[3]. [1]Research Core for Interdisciplinary Sciences (RCIS), Okayama University, Okayama, Japan, [2]Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu 292-0818, Japan, [3]RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan

PS11-419. **Effect of monoterpene, sabinene, to rice pathogens.** S. Miyoshi[1], K. Kohzaki[1], Y. Kokudo-Yamasaki[1], Y. Hosokawa-Shinonaga[1], K. Akimitsu[1], K. Gomi[1]. [1]Faculty of agriculture, Kagawa University, Kagawa, Japan

PS11-420. **Exploiting the priming ability of *Thellungiella salsuginea* to improve biotic and abiotic stress tolerance.** M. J. Champigny[1], V. Catana[1], W. Sung[1], M. Yeo[1], M. Macleod[1], P. Summers[1], B. Golding[1], R. Cameron[1], E. Weretilnyk[1]. [1]Department of Biology, McMaster University, Hamilton Ontario, Canada

PS11-421. **Suppression of cucumber diseases by using the spent mushroom substrate of *Lyophyllum decastes* and *Pleurotus eryngii*.** R. Y. Parada[1], S. Murakami[2], N. Shimomura[1], H. Otani[1]. [1]Faculty of Agriculture, Tottori University, Tottori, Japan, [2]The Tottori Mycological Institute

PS11-422. **Studies on potential roles of sulfur compounds for diseases control in the oriental pear orchard.** K.-H. Min[1], J.-P. Ryu[1], S.-H. Lee[1], W.-S. Kim[1], B. H. Cho[1], K.-Y. Yang[1]. [1]Korean Pear Export Research Organization, Department of Plant Biotechnology (BK21 program), College of Agriculture and Life Science, Chonnam National University, Gwangju, Korea

PS11-423. **Screening of metabolites derived from soil microorganisms for induction of plant resistance against *Tobacco mosaic virus*.** T. Kuan[1], H.-H. Yeh[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan (R.O.C.)

PS11-424. **Ethylene Response Factor (ERF) transcription factors of the B-3 subgroup include master regulators of ethylene signaling and mediate resistance to root pathogens without adversely affecting rhizobial symbiosis.** J. P. Anderson[1,2], J. Lichtenzveig[1,3], L. Onate-Sanchez[1,4], K. B. Singh[1,2]. [1]CSIRO Plant Industry, Floreat, Western Australia, [2]The UWA

Institute of Agriculture, The University of Western Australia, WA, Australia, [3]Department of Environment and Agriculture, Curtin University of Technology, Bentley, WA, Australia, [4]Current address: Centro de Biotecnología y Genómica de Plantas, Madrid, Spain

PS11-425. **Broad-spectrum disease resistance by *BSR1* shares transcriptional components with BTH-inducible resistance in rice.** S. Maeda[1], S. Sugano[1], M. Nakagome[1], A. Miyao[1], C.-J. Jiang[1], H. Takatsuji[1], M. Mori[1]. [1]National Institute of Agrobiological Sciences

PS11-426. **Determination of R gene specificity in bread wheat.** R. Kronbak[1], C. R. Ingvardsen[1], S. Walter[2], M. S. Hovmoeller[2], P. B. Holm[1], H. Brinch-Pedersen[1], P. L. Gregersen[1]. [1]Department of Molecular Biology and Genetics, Science and Technology, Aarhus University, Denmark., [2]Department of Agroecology, Science and Technology, Aarhus University, Denmark.

PS11-427. **Visualisation of phylloplane biofilms using Episcopic Differential Interference Contrast (EDIC) microscopy and the investigation of nitric oxide for biofilm control at the spinach phylloplane.** N. Gibbins[1], J. S. Webb[1], C. W. Keevil[1]. [1]Centre for Biological Sciences, University of Southampton, Southampton, UK

PS11-428. **Physiological and enzymatic characterization of *Burkholderia* spp. isoloted from cadmium contaminated soil.** M. N. Dourado[1], P. F. Martins[1], T. Tezzoto[1], M. C. Quecine[1], R. A. Azevedo[1]. [1]Escola Superior de Agricultura Luiz de Queiroz, University of Sao Paulo, Piracicaba, Brazil, [2]University of Sao Paulo

Evolution of susceptibility and resistance

PS12-429. **Identification of a genetic factor determining the durability of a plant major resistance gene and quantitative resistance to virus accumulation.** J. Quenouille-Lederer[1,2], E. Paulhiac[1], P. Mistral[1], G. Nemouchi[1], A.-M. Sage-Palloix[1], B. Savio[1], V. Simon[2], B. Moury[2], A. Palloix[1]. [1]Unite de Genetique et Amelioration des Fruits et Legumes, INRA PACA, Avignon, France., [2]Unite de Pathologie Vegetale, INRA PACA, Avignon, France.

PS12-430. **Hexose oxidase provides red algae with a mechanism for attacking bacteria.** K. Ogasawara[1], N. Hatsugai[2]. [1]Laboratory of Molecular Biology, Graduate school of Agriculture, Hokkaido University, [2]Research Center for Cooperative Projects, Hokkaido University

PS12-431. **Identification and molecular mapping of a wheat gene for resistance to a *Polypogon* isolate of *Colletotrichum cereale*.** R. Mori[1], Y. Inoue[1], Y. Takahashi[1], S. Kiguchi[1], Y. Tosa[1]. [1]Graduate School of Agricultural Sciences, Kobe University, Kobe, Japan

PS12-432. **Identification and functional analysis of novel rice blast field resistance gene, *OsXK2b*.** T. Murakami[1], Y. Kobayashi[1], I. Kobayashi[1]. [1]Grad. Schl. of Region. Innova. Studies/ Life Sci. Res. Cntr, Mie Univ

PS12-433. **Evolution of the resistance against TMV in *Nicotiana* spp.** H. Kuang[1], F. Ren[1], S. Chen[1], J. Chen[1]. [1]Dept of Vegetable crops, Huazhong Agricultural University, Wuhan, China

PS12-434. **The chloroplast RECA1 is required for the immune response of Arabidopsis to bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000.** H. Jeon[1], J. Kwon[1], H. Lee[1], M. Kim[1]. [1]Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

PS12-435. **Purple acid phosphatase 5 is required for regulation of defense responses against *Pseudomonas syringae* pv. *tomato* in Arabidopsis.** S. Ravichandran[1], S. Stone[1], B. Prithiviraj[2]. [1]Department of Biology, Dalhousie University, [2]Deparment of Environmental Sciences, Nova Scotia Agricultural College

PS12-436. **The complete genome sequence of Southern rice black-streaked dwarf virus isolated from Vietnam.** T.-S. Dinh[1,2], C. Zhou[1], X. Cao[1], C. Han[1], J. Yu[1], D. Li[1], Y. Zhang[1]. [1]State Key Laboratory of Agro-Biotechnology, College of Biological Sciences, China Agricultural University, Beijing, China, [2]Faculty of Agriculture Forestry Fisheries, Vinh University, Vinh city, Nghe An province 42000, Vietnam

Plant response

PS13-437. **Cloning and characterization of a novel canonical rice resistance gene to *Xanthomonas oryzae* pv. *oryzae*.** Y. Chen[1], Z. He[1]. [1]Institute of Plant Physiology and Ecology, SIBS, CAS

PS13-438. **The GDSL/SGNH lipases OsGL1 and OsGL2 negatively regulate basal immunity in rice.** M. Gao[1], W. Yang[1], Z. He[1]. [1]Institute of Plant Physiology and Ecology, SIBS, CAS.

Shanghai, China

PS13-439. **A rice chitinase-like xylanase inhibitor protein, OsXI1, is related with antifungal activity and plant development.** D. Y. Lee[1], J. Wu[1], Y. Wang[2], S. G. Kim[2], S. T. Kim[3], K. Y. Kang[1,2]. [1]Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, South Korea,, [2]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, 660-701, South Korea,, [3]Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea

PS13-440. **Connecting pathogen perception to transcriptional reprogramming in plant immune responses.** N. A. Peine[1], A. V. Garcia[2], J. Bautor[1], J. E. Parker[1]. [1]Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany, [2]Plant Genomics Research, Unite de Recherche en Genomique Vegetale, Institut National de la recherche agronomique, Evry, France

PS13-441. **Leaf oil bodies produce an anti-fungal compound actively in dying tissues.** T. L. Shimada[1,2], Y. Takano[2], T. Shimada[1], M. Fujiwara[3], Y. Fukao[3], M. Mori[4], R. Sasaki[5,6], K. Aoki[5,6], I. Hara-Nishimura[1]. [1]Graduate School of Science, Kyoto University, Kyoto, Japan, [2]Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [3]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan, [4]Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Ishikawa, Japan, [5]Kazusa DNA Research Institute, Chiba, Japan, [6]CREST, JST, Japan

PS13-442. **Plant programmed cell death caused by an autoactive form of Prf is suppressed by co-expression of the Prf LRR domain.** X. Du[1], M. Miao[1], G. B. Martin[2], F. Xiao[1]. [1]Dept. of Plant, Soil and Entomological Sciences, University of Idaho, [2]Boyce Thompson Institute for Plant Research and Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA

PS13-443. **Carbon/nitrogen regulatory ubiquitin ligase ATL31 and ATL6 control the defense response in Arabidopsis.** S. Maekawa[1], S. Yasuda[1], T. Sato[1], J. Yamaguchi[1]. [1]Faculty of Science and Graduate School of Life Science, Hokkaido University, Sapporo, Japan

PS13-444. **Cloning of rice blast resistance gene *Pi34* and comparative analysis to explore a cue of durable resistance.** H. Kito[1], K. Zenbayashi-Sawata[1]. [1]Tohoku Agricultural Research Center, National Agriculture and Food Research Organization

PS13-445. **Qa-SNAREs localized to the trans-Golgi network regulate multiple transport pathways and extracellular disease resistance in plants.** T. Uemura[1], H. Kim[2], C. Saito[3], K. Ebine[1], T. Ueda[1], P. Schulze-Lefert[2], A. Nakano[1,3]. [1]Graduate School of Science, University of Tokyo, [2]Max Planck Institute for Plant Breeding Research, [3]RIKEN, ASI

PS13-446. **A pseudokinase under balancing selection confers quantitative and broad spectrum disease resistance in Arabidopsis.** C. Huard-Chauveau[1], M. Debieu[1], L. Perchepied[1], C. Glorieux[2], N. Faure[2], J. Bergelson[3], F. Roux[2], D. Roby[1]. [1]UMR CNRS-INRA Laboratory of Plant-Microorganism Interactions, [2]Laboratoire de Genetique et Evolution des Populations Vegetales, UMR CNRS 8198, Universite; des Sciences et Technologies; Lille 1, France, [3]Department of Ecology and Evolution, University of Chicago, 1101 E. 57th Street, Chicago, IL 60637, USA

PS13-447. **Characterization of constitutively active OsRac1 (CA-gOsRac1) transgenic rice plants generated by gene targeting.** T. T. Dang[1], S. Zenpei[2], R. Terada[2], Y. Kawano[1], K. Shimamoto[1]. [1]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, Nara, Japan, [2]Meijo University, Nagoya, Japan

PS13-448. **Geraniol synthase whose mRNA is induced by host-selective ACT-toxin in the ACT-toxin-insensitive rough lemon (*Citrus jambhiri*).** H. Shishido[1], Y. Miyamoto[1], R. Ozawa[2], Y. Kokudo-Yamasaki[1], S. Taniguchi[1], J. Takabayashi[2], K. Akimitsu[1], K. Gomi[1]. [1]Faculty of Agriculture, Kagawa University, Kagawa, Japan, [2]Center for Ecological Research, Kyoto University, Otsu, Shiga, Japan

PS13-449. **A novel transcription factor, ANAC042, involved in the regulation of camalexin biosynthesis in *Arabidopsis*.** D. Ohta[1], H. Saga[1], T. Ogawa[1]. [1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University

PS13-450. **The anticipation of danger: MAMP perception enhances AtPep-triggered oxidative burst.** D. Klauser[1], S. Bartels[1], P. Flury[2], T. Boller[1]. [1]The Botanical Institute, University of Basel, Basel, Switzerland, [2]Institute of Integrative Biology, Plant Pathology, Swiss Federal Institute of Technology, Zurich, Switzerland

PS13-451. **Imaging analysis of mitochondrial movement in rice cells during rice *Magnaporthe oryzae* interactions.** S. Mochizuki[1,2], K. Saitoh[1], Y. Kubo[2], E. Minami[1], Y. Nishizawa[1]. [1]National Institute of Agrobiological Sciences, Tsukuba, Japan, [2]Kyoto Prefectural University, Kyoto,

Japan

PS13-452. **PAMP-mediated pathogen defense in *Solanum tuberosum*.** R. Landgraf[1], S. Altmann[1], L. Eschen-Lippold[1], S. Sonnewald[2], U. Sonnewald[2], S. Rosahl[1]. [1]Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany, [2]University of Erlangen, Staudtstr. 5, 91058 Erlangen, Germany

PS13-453. **Climate change effects on the interaction between barley and two fungal pathogens with opposite lifestyles.** B. L. Mikkelsen[1], C. G. Rayapuram[1], M. Lyngkjaer[1]. [1]Department of Plant Biology and Biotechnology, University of Copenhagen, Copenhagen, Denmark

PS13-454. ***Medicago truncatula* as a model to study vascular wilt disease: genetic traits and regulatory mechanisms.** C. Ben[1,2], M. Toueni[1,2], S. Montanari[1], S. Amatya[1], A. Negahi[1,2], G. Mathieu[1], M.-C. Gras[3], D. Noel[4], L. Gentzbittel[1,2], M. Rickauer[1,2]. [1]Universite de Toulouse, INP, ENSAT, Laboratoire d'Ecologie Fonctionnelle, [2]CNRS, UMR 5245EcoLab ; 31326 Castanet-Tolosan, France, [3]Societe R2n Groupe RAGT, 12510 Druelle, France, [4]Tourneur Barenbrug Recherches, Negadis, 82600 Mas-Grenier, France

PS13-455. **Cross-talk between *AtNHR2A* and *AtNHR2B* to modulate nonhost defense responses in *Arabidopsis*.** C. M. Rojas[1], S. Lee[1], M. Senthil-Kumar[1], A. Kaundal[1], H.-K. Lee[1], K. S. Mysore[1]. [1]The Samuel Roberts Noble Fundation, Ardmore, OK, USA.

PS13-456. **ROS, a two-faced Janus in plant responses to pathogens?** S. Siddique[1], C. Matera[1], M. Sobczak[2], S. Hasan[1], P. Gutbrod[1], F. M. W. Grundler[1]. [1]INRES, Department of Molecular Phytomedicine, University of Bonn, [2]Department of Botany; Warsaw University of Life Sciences (SGGW); Warsaw Poland

PS13-457. **A sec14P phospholipids transfer protein regulates plant immunity in *Nicotiana* plants.** A. Kiba[1,2,3], K. Ohnishi[2], H. Yoshioka[3], Y. Hikichi[1]. [1]Faculty of Agriculture Kochi University, [2]Rearch Institute of Molecular Genetics Kochi University, [3]Graduate School of Agriculture Nagoya University

PS13-458. **Functional analysis of the elicitor-inducible bZIP transcription factor OsTGAP1 in rice.** K. Miyamoto[1], T. Matsumoto[2], K. Komiyama[1], A. Okada[1], T. Chujo[1], H. Yoshikawa[2,3], N. Shibuya[4], H. Nojiri[1], H. Yamane[5], K. Okada[1]. [1]Biotechnology Research Center, The University of Tokyo, Tokyo, Japan, [2]Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture, Japan, [3]Department of Bioscience, Tokyo University of Agriculture, Japan, [4]Department of Life Sciences, Faculty of Agriculture, Meiji University, Japan, [5]Department of Biosciences, Teikyo University,Japan

PS13-459. **Molecular mapping of *Rmo2*, a core locus conditioning the resistance of barley to various host-specific subgroups of *Magnaporthe oryzae*.** G.-S. Hyon[1], N. T. T. Nga[1], K. Sato[2], I. Chuma[1], K. Okada[3], Y. Inoue[1], Y. Tosa[1]. [1]Graduate School of Agricultural Sciences, Kobe University, [2]2Institute of Plant Science and Resources (IPSR), Okayama University, [3]3National Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO)

PS13-460. ***Arabidopsis* NSL2-related cell death is induced by ROS production from chloroplasts.** Y. Maruyama[1], J. Yamaguchi[1]. [1]Faculty of Science and Graduate School of Life Science, Hokkaido University, Sapporo, Japan

PS13-461. **Application of D-allose for disease control of rice bakanae disease: Sugar phosphorylation of D-allose by hexokinase gives GA-dependent shoot growth inhibition.** T. Fukumoto[1], K. Ohtani[1], A. Kano[1], S. Tajima[1], K. Izumori[1], T. Ohara[2], Y. Shigematsu[2], T. Ohkouchi[2], Y. Ishida[3], Y. Tada[1], K. Ichimura[1], K. Gomi[1], K. Akimitsu[1]. [1]Faculty of Agriculture, Rare Sugar Research Center, and Gene Research Center, Kagawa University, Miki, Kagawa, 761-0795, Japan, [2]Mitsui Chemicals Agro Inc, Agrochemicals Research Center, 1358, Ichimiyake, Yasu, Shiga 520-2362, Japan, [3]Shikoku Research Institute Inc., Yashima-nishi, Takamatsu, 761-0192, Japan

PS13-462. **Overexpression of tobacco Dof transcription factor enhances transcriptional activation of the virus resistance gene *N* and ROS generation.** M. Takano[1], Md. A. Haque[1], N. Sasaki[1], H. Nyunoya[1]. [1]Gene Research Center, Tokyo University of Agriculture and Technology, Tokyo, Japan

PS13-463. **Phosphorylated D-allose confers disease resistance with ROS generation in Rice.** A. Kano[4], T. Fukumoto[1], K. Ohtani[1], A. Yoshihara[1], T. Ohara[2], S. Tajima[1], K. Izumori[1], Y. Shigematsu[2], K. Tanaka[2], T. Ohkouchi[2], Y. Ishida[3], Y. Nishizawa[4], K. Ichimura[1], Y. Tada[1], K. Gomi[1], K. Akimitsu[1]. [1]Faculty of Agriculture, Rare Sugar Research Center, and Gene Research Center, Kagawa University, Miki, Kagawa, Japan, [2]Mitsui Chemicals Agro Inc, Agrochemicals

Research Center, Ichimiyake, Yasu, Shiga, Japan, [3]Shikoku Research Institute Inc., Yashima-nishi, Takamatsu, Kagawa, Japan, [4]National Institute of Agrobiological Sciences, Tsukuba, Japan

PS13-464. **Isolation and identification of natural diterpenes that inhibit bacterial wilt disease in tobacco, tomato, and *Arabidopsis* and analysis of their mode of action.** S. Seo[1], K. Gomi[2], H. Abe[3], M. Kobayashi[4], H. Seto[3,7], Y. Ichinose[6], I. Mitsuhara[1], Y. Ohashi[1]. [1]Plant-Microbe Interactions Research Units, National Institute of Agrobiological Sciences, Tsukuba, Japan, [2]Kagawa University, Kagawa, Japan, [3]RIKEN, Tsukuba, Japan, [4]National Institute of Floricultural Science, Tsukuba, Japan, [5]National Agricultural Research Center, Tsukuba, Japan, [6]Okayama University, Okayama, Japan, [7]RIKEN, Wako, Japan

PS13-465. **Dispersed benzoxazinone gene cluster: Molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye.** M. Sue[1], C. Nakamura[1], H. Nakashita[1]. [1]Tokyo University of Agriculture

PS13-466. **Identification of molecules that modulate pathogen induced programmed cell death in *Arabidopsis*.** A. K. Nilsson[1], M. X. Andersson[1], L. Adolfsson[1], O. Johansson[1], F. Pinosa[1], C. Garcia[1], M. Tor[2], M. Hamberg[3], M. Ellerstrom[1]. [1]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden, [2]National Pollen and Aerobiology Research Unit, Institute of Science and the Environment, University of Worcester, Worcester, England, [3]Division of Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

PS13-467. **Ergosterol perception in plant systems: an "omics" approach.** L. A. Piater[1], F. Tugizimana[1], J. S. Sherwood[1], R. L. Klemptner[1], I. A. Dubery[1]. [1]Department of Biochemistry, University of Johannesburg, Johannesburg, South Africa

PS13-468. **Proteasome transformation in response to pathogen attack.** T. Sato[1], H. Sun[1], S. Maekawa[1], S. Yasuda[1], M. Fujiwara[2], Y. Fukao[2], J. Yamaguchi[1]. [1]Hokkaido University, [2]Nara Institute of Science and Technology

PS13-469. **MAMP-responsive phosphoprotein RAM1 negatively regulates ROS production in Arabidopsis.** H. Matsui[1], Y. Nomura[1], H. Nakagami[1]. [1]Plant Proteomics Research Unit, Plant Science Center, RIKEN Yokohama Institute, Kanagawa, Japan

PS13-470. **Plants growth promotion by *Streptomyces*. ll. Involvement of plant pathway.** H.-Y. Yang[1], C. W. Chen[1]. [1]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University

PS13-471. **Plants growth promotion by *Streptomyces*. I. Involvement of polyphenol oxidases of *Streptomyces*.** C. W. Chen[1], H.-Y. Yang[1]. [1]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University

PS13-472. **MAPK cascades control *NbRBOHB* promoter activity in *Nicotiana benthamiana*.** T. Nakano[1], N. Miyagawa[1], M. Yoshioka[1], N. Ishihama[1], H. Yoshioka[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

PS13-473. **Molecular characterization and regulation of a Nicotiana tabacum S-domain receptor-like kinase gene induced during an early rapid response to lipopolysaccharides.** I. A. Dubery[1], N. Sanabria[1], H. Van Heerden[1]. [1]Department of Biochemistry, University of Johannesburg, Aucland Park, South Africa

PS13-474. **The role of a splice variant product of the virus resistance gene *N* in the induction of hypersensitive response.** M. Takaoka[1], M. Takano[1], Md. A. Haque[1], N. Sasaki[1], H. Nyunoya[1]. [1]Gene Research Center, Tokyo University of Agriculture and Technology, Tokyo, Japan

PS13-475. **Mapping PAMP Responses in *Brassicas*.** S. R. Lloyd[1], H. Schoonbeek[1], C. Zipfel[2], C. Ridout[1]. [1]The John innes Centre, [2]The Sainsbury Laboratory

PS13-476. **Inception of infection: the boon and the curse for *Pseudomonas syringae*.** S. Panchal[1], D. Roy[1], M. Melotto[1]. [1]Department of Biology, University of Texas at Arlington, TX, USA

PS13-477. **Leucine derived hydroxy nitrile glucosides in barley and their relation to powdery mildew infection.** P. S. Roelsgaard[1], C. E. Olsen[1], K. Joergensen[1], M. Lyngkjaer[1], B. L. Moeller[1]. [1]Plant Biochemistry, LIFE, University of Copenhagen

PS13-478. **RNA-seq analysis of the tomato immune response identifies genes whose expression is induced by MAMPs and suppressed by type III effectors.** H. G. Rosli[1], Z. Fei[1], Y. Zheng[1], A. R. Collmer[2], G. B. Martin[1,2]. [1]Boyce Thompson Institute for Plant Research, Ithaca, NY, USA, [2]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA

PS13-479. **Transcription factors, which connect MAMPs-responsive MAPK cascade to the coordinately-expressed phenylpropanoid biosynthesis genes.** M. Kishi-Kaboshi[1], A. Takahashi[1], H. Hirochika[1]. [1]National Institute of Agrobiological Sciences, Tsukuba, Japan

PS13-480. **Isolation and characterization of lms, a rice lesion mimic mutant with enhanced resistance to rice blast (Magnaporthe oryzae).** M. Tamiru[1], J. R. Undan[1,2], A. Abe[1,2,3], R. Fekih[1], R. Terauchi[1]. [1]Iwate Biotechnology Research Center, [2]United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, 020-8550, Japan, [3]Iwate Agricultural Research Center, Narita 20-1, Kitakami, Iwate 024-0003, Japan

PS13-481. **Functional characterization of *Arabidopsis WRKY55* gene in plant defense against a bacterial pathogen.** J.-P. Ryu[1], Y. K. Lee[1], K.-H. Min[1], B. H. Cho[1], K.-Y. Yang[1]. [1]Department of Plant Biotechnology (BK21 program), College of Agriculture and Life Science, Chonnam National University, Gwangju, Korea

PS13-482. **A dual Resistance-protein system confers resistance against fungal and bacterial pathogens.** Y. Narusaka[1], K. Shirasu[2], Y. Takano[3], M. Narusaka[1]. [1]Research Institute for Biological Sciences, Okayama, Okayama, Japan, [2]RIKEN PSC, [3]Grad. Sch. of Agri. Kyoto Univ.

PS13-483. **Breaking restricted taxonomic functionality by dual resistance genes.** M. Narusaka[1], K. Shirasu[2], Y. Kubo[3], T. Shiraishi[4], K. Hatakeyama[5], T. Hirai[6], K. Kawamoto[6], H. Ezura[6], Y. Nanasato[7], Y. Tabei[7], Y. Takano[8], Y. Narusaka[1]. [1]Research Institute for Biological Sciences, Okayama, Okayama, Japan, [2]RIKEN PSC, [3]Grad. Sch. Agri., Kyoto Pref. Univ., [4]Grad. Sch. Nat. Sci. & Tech., Okayama Univ., [5]Natl. Res. Inst. Veg. & Tea Sci., [6]Grad. Sch. Life Env. Sci., Tsukuba Univ., [7]Natl. Inst. Agr. Sci., [8]Grad. Sch. of Agri., Kyoto Univ.

PS13-484. **Microarray analysis of gene expression profiles induced by neutralized phosphorous acid and *Phytophthora parasitica* in tomato.** C.-H. Wu[1], C.-P. Lin[1], P.-J. Ann[2], R.-F. Liou[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 106, Taiwan, [2]Plant Pathology Division, Taiwan Agriculture Research Institute, Taichung 413, Taiwan

PS13-485. **Molecular mechanisms for disease resistance in rice that is regulated by the transcriptional activator OsWRKY53.** S. Ogawa[1], K. Miyamoto[1], T. Shimizu[1], Y. Masuda[1], T. Chujo[1], Y. Nishizawa[2], E. Minami[2], H. Nojiri[1], H. Yamane[3], K. Okada[1]. [1]Biotechnology Research Center, The University of Tokyo, Tokyo, Japan, [2]National Institute of Agrobiological Sciences, [3]Department of Biosciences, Teikyo University

PS13-486. **Characterization of a novel pathogenesis-related protein from *Solanum lycopersicum*.** S.-H. Yi[1], R.-F. Liou[1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

PS13-487. **The transcriptional response in potato to infection by *Pectobacterium carotovorum* subsp. *brasiliensis* and the role of coronafacic acid in manipulating plant defences.** P. Ramakrishnan[1], M. Fiers[2], P. Panda[1], A. Pitman[1,2]. [1]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [2]New Zealand Institute for Plant & Food Research, Private Bag 4704, Christchurch, New Zealand

PS13-488. **Gene expression analysis during acibenzolar-S-methyl induced systemic disease resistance in cucumber using cross species microarrays.** S. A. Deepak[1], H. Ishii[2]. [1]Genotypic Technology Pvt. Ltd., [2]National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan

PS13-489. **Regulation of hypersensitive response by translationally controlled tumor protein in *Nicotiana benthamiana*.** M. Gupta[1], K. Ohnishi[2], H. Mizumoto[1], Y. Hikichi[1], A. Kiba[1]. [1]Laboratory of plant Pathology and biotechnology, Kochi University, kochi, Japan, [2]Research Institute of Molecular genetics, Kochi University, Nankoku, Kochi

PS13-490. **NOD1, a negative regulator of plant immune response, is required for establishment of disease susceptibility during *Nicotiana benthamiana-Ralstonia solanacearum* interaction.** M. Nakano[1,2], M. Nishihara[3], K. Ohnishi[4], Y. Hikichi[2], A. Kiba[2]. [1]The United Graduate School of Agricultural Sciences, Ehime University, Ehime, Japan, [2]Faculty of Agriculture, Kochi University, Kochi, Japan, [3]Iwate Biotechnology Research Center, Iwate, Japan, [4]Reaserch Institute of Molecular Genetics, Kochi University, Kochi, Japan

PS13-491. **A positive regulatory role of the watermelon *ClWRKY70* gene for disease resistance in transgenic *Arabidopsis*.** K.-Y. Yang[1], S. M. Cho[2], B. H. Cho[1]. [1]Department of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, [2]Department of Floriculture, Chunnam Techno College, Jeonnam, Republic of Korea.

PS13-492. **The Arabidopsis anion channels participate in innate immunity.** W. Guo[1], X. Chen[1], C. Tian[1], C. Wu[1], H. Li[1], J.-L. Qiu[1]. [1]Institute of Microbiology, Chinese Academy of Science,

Beijing, China

PS13-493. **High-throughput screening of chili pepper proteases function in *Nicotiana benthamiana* following pathogens infections.** C. Bae[1], D. Lee[1], J. Kim[2], C.-G. Hur[2], D. Choi[1]. [1]Department of Plant Science, Seoul National University, Seoul, Korea, [2]Plants Systems Engineering Research Center, KRIBB, Daejeon, 305-805, Korea

PS13-494. ***Capsicum*-specific secreted protein CaSD1 has multiple roles in pathogen defense, delay of senescence, and trichome formation.** E. Seo[1], S.-I. Yeom[1], S. Jo[2], H. Jeong[1], B.-C. Kang[1], D. Choi[1]. [1]Department of Plant Science, Seoul National University, Seoul, Korea, [2]Seeders Inc., Daejeon, Korea

PS13-495. **Development of a high-throughput system to monitor pathogen-responsive gene expression in *Arabidopsis thaliana* seedlings using bioluminescent reporters.** M. Kusama[1], N. Urata[1], G. Banzashi[1], R. Ogura[2], S. Ogata[1], K. Hiratsuka[1]. [1]Graduate School of Environmental and Information Sciences, Yokohama National University, Kanagawa, Japan, [2]Venture Business Laboratory, Yokohama National University, Kanagawa, Japan

PS13-496. **The influence of infection pressure of *Synchytrium endobioticum* (Schilb.) Perc. on reaction of potato.** J. Przetakiewicz[1]. [1]Plant Breeding and Acclimatization Institute, National Research Institute, Department of Plant Pathology, Laboratory of Quarantine Organisms, Radzikow, Poland

PS13-497. **Suppression of autophagosome formation by cryptogein, a proteinaceous elicitor from an oomycete, in tobacco BY-2 cells.** M. Okada[1], S. Hanamata[1], T. Kurusu[1,2], K. Kawamura[1], K. Kuchitsu[1,2]. [1]Department of Applied Biological Science, Tokyo University of Science, Chiba, Japan, [2]Research Institute for Science and Technology (RIST), Tokyo University of Science, Chiba, Japan

PS13-498. **Roles of an S-type anion channel SLAC1 in the regulation of cryptogein-induced initial responses and hypersensitive cell death in tobacco BY-2 cells.** T. Kurusu[1,2], K. Saito[1], S. Horikoshi[1], S. Hanamata[1], J. Negi[3], K. Iba[3], K. Kuchitsu[1,2]. [1]Department of Applied Biological Science, Tokyo University of Science, Chiba, Japan, [2]Research Institute for Science and Technology (RIST), Tokyo University of Science, Chiba, Japan, [3]Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

PS13-499. **UV-B irradiation-induced suppression of necrotic symptom development and TSWV accumulation in tobacco plants.** M. Kobayashi[1], M. Yamada[2], M. Ishiwata[2], M. Satou[1], T. Hisamatsu[1]. [1]NARO Institute of Floricultural Science, [2]Panasonic Corporation

PS13-500. **Cloning and expression analysis of an arginine decarboxylase gene from bottle gourd (*Lagenaria siceraria*).** S. Kim[1], B. H. Cho[1], K.-Y. Yang[1]. [1]The Department of plant biotechnology, Chonnam national university, Gwang-ju, Korea

PS13-501. **A novel communication between plants and soil bacteria through volatile substances.** J. Murata[1], H. Komura[1]. [1]Suntory Foundation for Life Sciences

PS13-502. **Pathogen-induced ERF68 in tomato modulate production of reactive oxygen species that cause cell death and pathogen resistance.** A.-C. Liu[1], C.-P. Cheng[1]. [1]Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

PS13-503. **Regulation of elicitor-induced Ca^{2+} influx and phytoalexin production by a voltage-gated Ca^{2+} permeable channel OsTPC1 in rice.** K. Kuchitsu[1,2], H. Hamada[1], T. Kurusu[2], E. Okuma[3], Y. Murata[3], K. Okada[4], H. Yamane[4]. [1]Department of Applied Biological Science, Tokyo University of Science, Noda, Japan, [2]Research Institute for Science and Technology, Tokyo University of Science, Noda, Japan, [3]Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan, [4]Biotechnology Research Center, University of Tokyo, Tokyo, Japan

PS13-504. **HSP70 regulates Tabtoxinine-β-lactam-induced cell death in *Nicotiana benthamiana*.** M. Itoh[1], Y. Yamamoto[1], C.-S. Kim[1], K. Ohnishi[2], H. Mizumoto[1], Y. Hikichi[1], A. Kiba[1]. [1]Faculty of Agriculture, Kochi University, Nankoku, Japan., [2]Research Institute of Molecular Genetics, Kochi University, Nankoku, Japan.

PS13-505. **Photosynthesis-mediated activation of PAMP-induced biosynthesis of salicylic acid in *Arabidopsis*.** T. Shiina[1], K. Nakai[1], D. Tojo[1], S. Sano[1], Y. Nakahira[1]. [1]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University

PS13-506. **Molecular analysis of rice *heme activator protein* (*OsHAP2E*) and *aspartic protease* (*OsAP77*) genes in response to biotic and abiotic stresses.** Md. M. Alam[1], H. Nakamura[3], H. Ichikawa[2], K. Kobayashi[1], N. Yamaoka[1], M. Nishiguchi[1]. [1]Faculty of Agriculture, Ehime University, Matsuyama, Ehime, Japan, [2]National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki, Japan, [3]Department of Applied Biological Chemistry, Graduate School of Agricultural Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo,

Japan

PS13-507. **Tryptophan-derived metabolites in the immunity of Brassicaceae species.** M. Pislewska-Bednarek[1,2], K. Kulak[1], E. Ver Loren van Themaat[2], P. Schulze-Lefert[2], P. Bednarek[1,2]. [1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, [2]Max Planck Institute for Plant Breeding Research, Cologne, Germany

PS13-508. **Salicylic acid induces genes for the unfolded protein response depending on IRE1 and bZIP60 in *Arabidopsis*.** Y. Nagashima[1], K. Mishiba[1], N. Koizumi[1]. [1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan

PS13-509. **Toward understanding of spatial and temporal regulation of hypersensitive response upon R-Avr recognition.** S. Betsuyaku[1,2], H. Urawa[3], Y. Kamei[3], K. Okada[3], H. Fukuda[2]. [1]Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan, [2]Graduate School of Science, University of Tokyo, [3]National Institute for Basic Biology

PS13-510. **Two histone-modifying proteins regulate plant immune response against *Pseudomonas* infection.** H. W. Jung[1,2], M. J. Kim[1], J. K. Park[2]. [1]Department of Genetic Engineering, Dong-A University, Busan, Korea, [2]Department of Medical Bioscience, Dong-A University, Busan, Korea

PS13-511. **Role of ceramidases in *Arabidopsis* morphogenesis and disease resistance.** J. Wu[1], Z. Liu[1], Z. Chang[1], J. Li[1], N. Yao[1]. [1]State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

PS13-512. **Several EAR motif-containing ERFs in group VIII are involved in HR cell death induction.** T. Ogata[1], Y. Matsushita[1]. [1]Gene Research Center, Tokyo University of Agriculture and Technology, Tokyo , Japan

PS13-513. **Bacteria induce systemic acquired resistance in barley.** S. Dey[1], M. Wenig[1], C. Knappe[1], A. C. Vlot[1]. [1]Institute of Biochemical Plant Pathology, Helmholtz Zentrum Muenchen

PS13-514. **The transcriptome of *Verticillium dahliae*-infected *Nicotiana benthamiana* determined by deep RNA sequencing.** L. Faino[1], R. de Jonge[1], B. Thomma[1]. [1]Phytopathology,WUR,Wageningen,The Netherlands

PS13-515. **The bilateral role of light in plant-pathogen interaction.** K. K. Aho[1], T. Kariola[1], T. E. Palva[1], H. Saarilahti[1]. [1]The Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

PS13-516. **Functional analysis of the interaction between *Puccinia psidii* and Eucalyptus grandis.** D. Moon[1], T. F. Leite[1], M. C. Quecine[1], A. C. M. Lima[1], L. M. Franceschini[1], C. A. Labate[1]. [1]Laboratorio Max Feffer de Genetica de Plantas, Departamento de Genetica, ESALQ-USP, Piracicaba, Brazil

PS13-517. **Regulation of intracellular redox by glyceraldehyde-3-phosphate-dehydrogenase during plant innate immunity.** E. M. Henry[1], J. Liu[1], J. M. Elmore[1], G. Coaker[1]. [1]Department of Plant Pathology, University of California at Davis, Davis, California, USA

PS13-518. **Transcriptome analysis of rice leaf and root cells inoculated with *Magnaporthe oryzae*.** S. Tanabe[1], Y. Fujisawa[1], M. Kimura[1], Y. Nishizawa[1], E. Minami[1]. [1]National Insutitute of Agrobiological Sciences, Ibaraki, Japan

PS13-519. **Different responses to FliCs of soft-rot pathogens is attributed to plant species and their sequence composition containing flg22 homologous region.** T. Ono[1], H. Yamamoto[1], M. Fujishiro[1], O. Netsu[1], S. Tsuyumu[1], H. Hirata[1]. [1]Faculty of Agriculture, Shizuoka University, Shizuoka, Japan

PS13-520. **Function of tomato *ERF36* and *ERF39* in response to bacterial wilt and abiotic stress.** S. Chong[1], T.-L. Yang[1], C.-P. Cheng[1]. [1]Graduate Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

PS13-521. **Functional study of tomato *ERF35* and *ERF38*.** Y.-J. Wang[1], T.-L. Yang[1], C.-P. Cheng[1]. [1]Graduate Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

Pathogenic bacteria / phytoplasma

PS14-522. **Cell-cell sinaling regulates common and contrasting traits in *Xanthomonas oryzae* pv. *oryzae*.** B. B. Pradhan[1], R. Rai[1], M. Ranjan[1], S. Chatterjee[1]. [1]Centre for DNA Fingerprinting and Diagnostics

PS14-523. **Tzs is involved in *Agrobacterium* virulence and growth.** H.-H. Hwang[1,2], Y.-L. Lee[1], Y.-H.

Li[1], F.-J. Yang[1], T.-F. Cheng[1], Y.-L. Tsai[2], E.-M. Lai[2]. [1]Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan, [2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

PS14-524. **Integrated management of citrus canker disease caused by *Xanthomonas citri* subsp. *citri* in Saudi Arabia.** A. Widyawan[1], Y. Y. Molan[1]. [1]King Saud University

PS14-525. **Secretome analysis of rice bacterial blight, *Xanthomonas oryzae* pv. *oryzae*.** S. G. Kim[1], Y. Wang[1], S. T. Kim[2], J. Wu[3], K. Y. Kang[1,3]. [1]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, South Korea, [2]Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea, [3]Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, 660-701, South Korea

PS14-526. **Genomic era of the model soft rot phytopathogen *Pectobacterium* sp. SCC3193 provides surprises in the collection of virulence determinants and the phylogenetic diversity of potato pathogenic soft rot bacteria.** J. S. Nykyri[1], O. Niemi[2], P. Koskinen[2], J. Nokso-Koivisto[3], M. Pasanen[1], M. Broberg[1,2], I. Plyusnin[3], P. Toronen[3], L. Holm[2,3], M. Pirhonen[1], E. T. Palva[2]. [1]Department of Agricultural Sciences, Plant Pathology, University of Helsinki, Helsinki, Finland, [2]Department of Biosciences, Division of Genetics, P.O. Box 56 (Viikinkaari 5), FI-00014 University of Helsinki, Finland, [3]Institute of Biotechnology, P.O. Box 56 (Viikinkaari 5), FI-00014 University of Helsinki, Finland

PS14-527. **Dissociation of bacterial population: a strategy of effective plant colonization.** V. Y. Gorshkov[1], A. G. Daminova[1], M. V. Ageeva[1], O. E. Petrova[1], P. V. Mikshina[1], N. E. Gogoleva[1], Y. V. Gogolev[1]. [1]Kazan Institute of Biochemistry and Biophysics, Kazan Research Centre, Russian Academy of Sciences, Kazan, Russia

PS14-528. **Acid-induced ExoR degradation derepresses the ChvG/ChvI two-component system to activate type VI secretion in *Agrobacterium tumefaciens*.** C.-F. Wu[1,2], J.-S. Lin[1], G.-C. Shaw[2], E.-M. Lai[1]. [1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, [2]Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan

PS14-529. **Identification of two new type-III effectors in *Xanthomonas oryzae* pv. *oryzicola* required for pathogenesis in rice.** L. Zou[1]. [1]School of Agriculture and Biology, Shanghai Jiao Tong University

PS14-530. **A highly-conserved single-stranded DNA-binding protein in *Xanthomonas* Works as a PAMP for PTI.** G. Chen[1], Y.-Z. Che[1], Y.-R. Li[1], W.-X. Ma[1], L.-F. Zou[1], H.-S. Zou[1]. [1]School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

PS14-531. **Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in Arabidopsis plants.** A. M. MacLean[1], A. Sugio[1], O. V. Makarova[2], K. C. Findlay[1], V. M. Grieve[1], R. Toth[3], M. Nicolaisen[2], S. A. Hogenhout[1]. [1]Cell & Developmental Biology, John Innes Centre, Norwich, United Kingdom, [2]Aarhus University, Department of Agroecology, Plant Pathology and Entomology, Slagelse, Denmark, [3]Max Planck Institute for Plant Breeding Research, Department of Plant Developmental Biology, Cologne, Germany

PS14-532. **Hcp2, a secreted protein of the phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000, is required for competitive fitness against bacteria and yeasts.** M. Haapalainen[1], H. Mosorin[2], F. Dorati[3], R.-F. Wu[4], E. Roine[2], S. Taira[2], R. Nissinen[1], L. Mattinen[1], R. Jackson[3], M. Pirhonen[1], N.-C. Lin[4]. [1]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland, [2]Department of Biosciences, University of Helsinki, Helsinki, Finland, [3]School of Biological Sciences, University of Reading, Reading RG6 6AJ, UK, [4]Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan R. O. C.

PS14-533. **Virulence determinants of the cucurbit pathogenic bacterium *Acidovorax citrulli*.** S. Burdman[1], N. Levi[1], O. Bahar[1], T. Zimmermann[1], T. Rosenberg[1], A. Castro-Sparks[2], R. Walcott[2], S. M. Traore[3], B. Zhao[3], G. Welbaum[3], J. Sikorski[4]. [1]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israel, [2]Department of Plant Pathology, University of Georgia, Athens GA, USA, [3]Department of Horticulture, Virginia Tech, Blacksburg VA, USA, [4]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

PS14-534. **Global genes expression profiling of *Xanthomonas axonopodis* pv. *glycines* 12-2 during infection in soybean.** T. Chatnaparat[1], S. E. Lindow[2], S. Prathuangwong[1]. [1]Department of Plant Pathology, Kasetsart University, Bangkok, Thailand, [2]Department of Plant and Microbial Biology, University of California, Berkeley, USA

PS14-535. **Transcriptional control of Arabidopsis responses to the pathogenic effector protein AvrRpm1 from *Pseudomonas syringae*.** O. Kourtchenko[1], E. Kristiansson[2], A. K. Nilsson[3], O. N. Johansson[3], A. Czihal[4], M. X. Andersson[3], D. Mackey[5], H. Baeumlein[3,4], M. Ellerstrom[3].

[1]Department of Chemistry and Molecular Biology, Gothenburg University, [2]Department of Mathematical Statistics, Chalmers University of Technology, [3]Department of Biological- and Environmental Sciences, Gothenburg University, [4]Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, [5]Department of Plant Cellular and Molecular Biology, Ohio State University

PS14-536. **Specific induction mechanism of rice immune responses by flagellins from *Acidovorax avenae*.** H. Hirai[1], Y. Uno[1], F.-S. Che[1]. [1]Graduate School of Biosciences, Nagahama Institute of Bio-Science and Technology

PS14-537. **The role of two-component response regulator in biofilm formation and pathogenicity by *Xanthomonas axonopodis* pv. *citri*.** T.-P. Huang[1], K.-M. Lu[1]. [1]Department of Plant Pathology, National Chung-Hsing University, Taiwan

PS14-538. **Involvement of a phosphinothricin N-acetyltransferase gene in virulence diversity of *Pseudomonas cichirii* strain SPC9018.** M. Tanaka[1], W. Md. Ullah[1], H. Mizumoto[1], K. Ohnishi[2], A. Kiba[1], Y. Hikichi[1]. [1]Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan, [2]RIMG, Kochi University, Kochi, Japan

PS14-539. **Involvement of a lectin gene, *fml*, in virulence of *Ralstonia solanacearum* strain OE1-1.** Y. Mori[1], N. Shiba[1], H. Mizumoto[1], K. Ohnishi[2], A. Kiba[1], Y. Hikichi[1]. [1]Lboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan, [2]RIMG, Kochi University, Kochi, Japan

PS14-540. **Functional roles of VirB2 in the type IV secretion system, T-pilus, and virulence of *Agrobacterium tumefaciens*.** H.-Y. Wu[1,2], C.-Y. Chen[2], J. Sheen[3,4], E.-M. Lai[1,2]. [1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, [2]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, [3]Department of Molecular Biology and Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston, MA 02114, USA, [4]Department of Genetics, Harvard Medical School, Boston, MA 02114, USA

PS14-541. **Hemin transported protein of *Xanthomonas axonopodis* pv. *glycines* functions on leaf colonization and virulence on soybean.** S. Prathuangwong[1], D. Athinuwat[2], W. Chuaboon[1], L. Kladsuwan[1], T. Chatnaparat[1]. [1]Department of Plant Pathology, Kasetsart University, Bangkok, Thailand, [2]Major of Organic Farming Management, Faculty Science and Technology, Thammasat University

PS14-542. **Biosynthesis of diffusible signal factor (DSF) signals in *Xanthomonas campestris* pv. *campestris* is induced by host metabolites.** Y. Deng[1], C. Chang[1]. [1]Institute of Molecular and Cell Biology, Singapore

PS14-543. **Functional characterization of genes encoding HD-GYP domain proteins in *Xanthomonas oryzae* pv *oryzicola*.** Y. Zhang[1], L. Wang[1], W. Jiang[1], D. Jin[1], M. Dow[2], W. Sun[1]. [1]Department of Plant Pathology, China Agricultural University, Beijing, China, [2]BIOMERIT Research Centre, Department of Microbiology, BioSciences Institute, University College Cork, Ireland

PS14-544. **Interactions of HrpB proteins in *Xanthomonas oryzae* pathovar *oryzae*.** H. Cho[1], E.-S. Song[1], I. Hwang[2], B.-M. Lee[1]. [1]National Academy of Agricultural Science, Rural Development Administration, Suwon, Korea, [2]Department of Agricultural Biotechnology and Center for Agricultural biomaterials, Seoul National University, , Seoul, 151-921, Korea

PS14-545. **Substrate specificity switching during type III secretion in the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria*.** J. Hausner[1], S. Schulz[1], C. Lorenz[2], N. Hartmann[1], D. Buettner[1]. [1]Department of Genetics, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany, [2]Harvard Medical School Microbiology, Boston, USA

PS14-546. **Variations in type III effector repertoires do not correlate with differences in pathological phenotypes and host range observed for *Xanthomonas citri* pv. *citri* pathotypes.** A. Escalon[1], S. Javegny[1], K. Vital[1], C. Verniere[1], L. Noel[2], O. Pruvost[1], M. Arlat[2,3], L. Gagnevin[1]. [1]CIRAD-Universite de la Reunion, St Pierre, Reunion Island, France, [2]Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR CNRS-INRA 2594/441, F-31320 Castanet-Tolosan, France., [3]Universite de Toulouse, F-31062 Toulouse, France.

PS14-547. **Regulons of *expA* and *rsmA* in *Pectobacterium* strain SCC3193.** M. E. Broberg[1]. [1]Department of Biosciences, University of Helsinki, Helsinki, Finland

PS14-548. **Quorum sensing mechanism mediates virulence control in the plant pathogen *Xylella fastidiosa*.** M. Ionescu[1], E. Beaulieu[1], C. Baccari[1], S. Chatterjee[1], N. Wang[1,2], N. Killiny[2], R.

P. P. Almeida[2], S. E. Lindow[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley, USA, [2]Department of Environmental Science, Policy and Management, University of California, Berkeley, USA

PS14-549. **Identifying factors involved in pathogenicity of *Ralstonia solanacearum* strains at low temperatures using a proteomics approach.** A. M. Bocsanczy[1], U. C. Achenbach[2], A. Mangravita-Novo[3], D. J. Norman[1]. [1]Department of Plant Pathology, University of Florida, MREC/IFAS, Apopka, Florida, U.S.A., [2]Development Lead North-East Europe. Syngenta Agro GmbH Am, Maintal, Germany, [3]Sanford-Burnham Medical Research Institute, Orlando, Florida, U.S.A.

PS14-550. **Experimental evolution of host specificity in *Pseudomonas syringae*.** H. C. McCann[1], P. B. Rainey[2], D. S. Guttman[1]. [1]Dept. of Cell & Systems Biology, University of Toronto, [2]New Zealand Institute for Advanced Study, Massey University, New Zealand

PS14-551. **N-acetyl-L-cysteine prevents *Xylella fastidiosa* colonization in citrus plant, thereby decreasing virulence.** T. E. Giorgiano[1], H. Della Coletta Filho[1], J. Pires Tomaz[1], M. A. Takita[1], M. A. Machado[1], A. Alves de Souza[1]. [1]Centro APTA Citros Sylvio Moreira, Cordeiropolis, Sao Paulo, Brasil

PS14-552. **Depriving sugars from apoplast located bacterial pathogens by regulating nutrient efflux is a plant defense strategy and is a component of nonhost resistance.** M. Senthil-Kumar[1], A. C. Srivastava[1], Y. Zhang[1], K. S. Mysore[1]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402 U.S.A.

PS14-553. **Type IV pilin is glycosylated in *Pseudomonas syringae* pv. *tabaci* 6605 and required for surface motility and virulence.** C. L. Nguyen[1], F. Taguchi[1], Q. T. Minh[1], Y. Inagaki[1], K. Toyoda[1], T. Shiraishi[1], Y. Ichinose[1]. [1]Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan

PS14-554. **A novel non-ribosomal peptide synthetase is required for pathogenicity of *Pectobacterium* on potatoes.** P. Panda[1], M. A. W. J. Fiers[2], K. Armstrong[1], T. Conner[3], A. R. Pitman[1,2]. [1]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [2]New Zealand Institute for Plant & Food Research, Lincoln, New Zealand, [3]AgResearch, Lincoln, New Zealand

PS14-555. **Identification and characterization of *Streptomyces* spp. causing potato common scab in Vietnam.** T. T. P. Nguyen[1], T. T. Nguyen[1], L. T. T. Nguyen[1]. [1]Department of Plant Biotechnology, Faculty of Biotechnology, Hanoi University of Agriculture, Vietnam

PS14-556. **Iron acquisition by phosphinothricin *N*-acetyltransferase-regulated siderophore may be one of determinants for virulence of *Pseudomonas cichorii*.** W. Md. Ullah[1], M. Tanaka[1], H. Mizumoto[1], K. Ohnishi[2], A. Kiba[1], Y. Hikichi[1]. [1]Lab. of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan, [2]RIMG, Kochi University

PS14-557. **Identification of novel effectors in *Pseudomonas syringae* pv. *actinidiae* the causal agent of kiwifruit canker.** M. D. Templeton[1,2], M. Fiers[3], A. Lu[3], E. H. A. Rikkerink[1], F. Bertels[4], B. Haubold[5], C. Brendolise[1], W. Cui[1], J. Rees-George[1], M. T. Andersen[1], H. McCann[4,6], J. Michtavy[4], P. Wang[6], D. Guttman[6], P. B. Rainey[4,5]. [1]Bioprotection, Plant and Food Reserch, Auckland New Zealand, [2]University of Auckland, Auckland, New Zealand, [3]Plant and Food Research, Lincoln, New Zealand, [4]NZAIS and Allan Wilson Centre, Massey University, Auckland, New Zealand, [5]Max Planck Institute for Evolutionary Biology, Plön, Germany, [6]University of Toronto, Toronto, Canada

PS14-558. **Transcriptional responses of *Pseudomonas syringae* to growth in epiphytic versus apoplastic leaf sites.** X. Yu[1], S. Lund[2], R. Scott[3], J. Williams[4], A. Records[4]. [1]Department of Plant Pathology and Microbiology, Iowa State University, Iowa, U.S., [2]Dept of Statistics, Iowa State University, U.S., [3]Dept of Plant and Microbial Biology, University of California-Berkeley, CA, U.S., [4]Dept of Plant Pathology and Microbiology, Texas A&M University, TX, U.S.

PS14-559. **Identification and characterization of new type III effectors from *Xanthomonas campestris* pv. *vesicatoria*.** S. Schulze[1], A. U. Singer[2,3], R. Szczesny[1], A. Krueger[1], F. Thieme[1], S. Thieme[1], A. Savchenko[2,3], U. Bonas[1]. [1]Department of Genetics, Martin Luther University Halle-Wittenberg, Halle , Germany, [2]Banting and Best Department for Medical Research, University of Toronto, C.H. Best Institute, Room 24, 112 College Street, Toronto, Ontario M5G 1L5, Canada, [3]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Walberg Street, Toronto, Ontario M5G 1L5, Canada

PS14-560. **XOO0635, a hybrid histidine kinase sensor of *Xanthomonas oryzae* pv. *oryzae*, is activated by sensing the low O_2 concentration and involved in stress tolerance and virulence.** Y. Kametani-Ikawa[1], A. Furutani[2], H. Ochiai[3], S. Tsuge[1]. [1]Laboratory of Plant pathology, Graduate School of Agriculture, Kyoto Prefectural University, [2]Gene Research Center, Ibaraki

University, [3]National Institute of Agrobiological Sciences

PS14-561. **The HSI-II gene cluster in *Pseudomonas syringae* pv. *tomato* DC3000 encodes a functional type VI secretion system, which is required for growth fitness in tomato and interbacterial competition.** N.-T. Hsu[1], C.-Y. Liu[1], Y.-C. Wang[1], C.-T. Sheu[1], Y.-H. How[1], N.-C. Lin[1]. [1]Department of Agricultural Chemistry, National Taiwan University,Taiwan, R.O.C.

PS14-562. **Aconitase B is required for optimal growth of *Xanthomonas campestris* pv. *vesicatoria* on pepper leaves.** J. Kirchberg[1], K. Wesolowska[1], G. Sawers[1]. [1]Institute of Microbiology, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany

PS14-563. **Identification of genes involved in *Ralstonia solanacearum* phage infection and LPS biogenesis.** C.-H. Li[1], K.-C. Wang[1], Y.-H. Hong[1], Y.-J. Chu[1], D.-K. Lu[1], W.-C. Yang[1], G. N. Gussin[2], I.-C. Chou[1], C. Cheng[1]. [1]Institute of Plant Biology, National Taiwan University, Taipei, Taiwan, [2]Department of Biology, The university of Iowa, Iowa City, IA, U. S. A.

PS14-564. **The *Clavibacter michiganensis* subsp. *michiganensis*-tomato interactome reveals perception of pathogen by host and suggests mechanisms of infection.** A. Savidor[1], D. Teper[1], K.-H. Gartemann[2], R. Eichenlaub[2], L. Chalupowicz[3], S. Manulis-Sasson[3], I. Barash[1], H. Tews[2], K. Mayer[2], R. J. Giannone[4], R. L. Hettich[4], G. Sessa[1]. [1]Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel, [2]University of Bielefeld, Bielefeld, Germany, [3]The Volcani Center, Bet Dagan, Israel, [4]The Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

PS14-565. **Molecular characterization of AvrBs3 from *Xanthomonas*.** T. Schreiber[1], D. Schmidt[1], A. Sorgatz[1], U. Bonas[1]. [1]Department of Genetics, Martin-Luther-University Halle-Wittenberg, Halle, Germany

PS14-566. ***Arabidopsis thaliana* as an experimental host for *Xylella fastidiosa*, causal agent of citrus variegated clorosis.** J. P. Tomaz[1], R. Caserta[1,2], M. A. Machado[1], A. A. de Souza[1]. [1]Instituto Agronomico de Campinas, Centro Apta Citros Sylvio Moreira, Cordeiropolis, São Paulo, Brazil, [2]Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

PS14-567. **Adaptation of *Pectobacterium atrosepticum* SCRI1043: to survive in order to infect.** O. E. Petrova[1], V. Y. Gorshkov[1], A. G. Daminova[1], M. V. Ageeva[1], N. E. Suzina[2], Y. V. Gogolev[1]. [1]Department: Laboratory of Molecular Biology, Kazan Institute of Biochemistry and Biophysics, Russian Academy of Science, Russian Federation, [2]Institute of Biochemisrty and Physiology of Microorganisms, Pushchino, Russian Federation

PS14-568. **Diversity of HrpL regulons in *Pseudomonas syringae* isolates.** T. S. Mucyn[1], A. L. Lind[1], S. Biswas[1], S. Yourstone[1], M. T. Nishimura[1], J. S. Cumbie[2], S. R. Grant[1], J. H. Chang[2], C. D. Jones[1], J. L. Dangl[1]. [1]University of North Carolina, Chapel Hill, US, [2]Oregon State University, Corvallis, US

PS14-569. **Differential expression of SU91-linked QTL in the interactions between common bean and strains of common bacterial blight pathogens.** W. Xie[1], S. McClymont[1], K. Yu[2], K. P. Pauls[1], A. Navabi[1,2]. [1]University of Guelph, [2]Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, 2585 County Road 20, Harrow, Ontario, Canada N0R 1G0

PS14-570. **Initial characterization of the two type VI secretion systems in *Pseudomonas syringae* pathovar *tomato* DC3000.** J. C. Valenta[1], L. M. Schechter[1]. [1]Department of Biology, University of Missouri-St. Louis, St. Louis, Missouri, USA

PS14-571. **Relationship between molecular diversity and HR induction in tobacco of Japanese strains of *Ralstonia solanacearum*.** K. Ohnishi[1], Y. Liu[1], L. Chen[1], Y. Zhang[1], A. Kanda[2], A. Kiba[2], Y. Hikichi[2]. [1]Research Institute of Molecular Genetics, Kochi University, Kochi, Japan, [2]Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan

PS14-572. ***Agrobacterium tumefaciens 6b* gene on T-DNA has activity of histone chaperon and represses expression of auxin-response genes in Arabidopsis.** S. Terakura[1,2], Y. Matsumura[1], H. Tagami[3], Y. Machida[1]. [1]Graduate School of Science, Nagoya University, Nagoya, Japan, [2]Takara-bio Inc., Japan, [3]Graduate School of Natural Sciences, Nagoya City University, Mizuho, Nagoya, Nagoya, Japan

PS14-573. **Diversity in *Erwinia amylovora* virulence on different *Malus* cultivars.** J. Pulawska[1], A. Mikicinski[1], A. Kuras[1], M. Lewandowski[1], P. Sobiczewski[1]. [1]Research Institute of Horticulture, Skierniewice, Poland

PS14-574. **Two avirulence effector genes of Japanese *Ralstonia solanacearum* strains are involved in**

pathogenicity to tobacco. L. Chen[1], Y. Zhang[2], A. Kiba[3], Y. Hikichi[3], K. Ohnishi[1]. [1]Research Institute of Molecular Genetics, Kochi University, Kochi, Japan, [2]Division of Microbiology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [3]Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan

PS14-575. **The role of *hrpRS* in regulation of virulence gene expression in *Pseudomonas syringae* pathovar tomato DC3000.** S. S. Thota[1], E. Loginicheva[1], J. L. Stauber[1], L. M. Schechter[1]. [1]Department of Biology, University of Missouri-St. Louis, St.Louis, Missouri, USA

PS14-576. **Differential expression of *in vivo* and *in vitro* protein profile of outer membrane of *Acidovorax avenae* subsp. *avenae*.** M. Ibrahim[1], Y. Shi[1], Q. Hui[1], A. Jabeen[1], L. Li[1], H. Liu[1], B. Li[1], M. Kube[1], G. Xie[1]. [1]Institue of Biotechnology, Zhejiang University, Hangzhou, China

Systems biology

PS15-577. **CFGP 2.0: A standard web-based bioinformatics portal for comparative and evolutionary genomics.** J. Choi[1], K. Cheong[1], G.-W. Lee[2], Y.-H. Lee[1]. [1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea, [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

PS15-578. **Laboratory Information Management System for functional genomics of *Magnaporthe oryzae*.** K. Jung[1,2], S. Kong[1,2], J. Park[1,2], S.-Y. Park[1,2], Y.-H. Lee[1,2]. [1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea, [2]Center for Fungal Genetic Resources, Center for Fungal Pathogenesis and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

PS15-579. **KNApSAcK Family Databases connect biological activities of metabolites and plants with microorganisms.** Y. Nakamura[1], F. M. Afendi[1], T. Katsuragi[1], S. Ikeda[1], K. Tanaka[2], A. H. Morita[1], Md. Altaf-Ul-Amin[1], S. Kanaya[1]. [1]Graduate School of Information Science, Nara institute of Science and Technology, Nara, Japan, [2]Institute of Natural Medicine, University of Toyama, Toyama, Japan

PS15-580. **Systems biology approach to study potato PVY interaction.** S. Baebler[1], K. Witek[2], T. Stare[1], M. Petek[1], K. Stare[1], M. Pompe-Novak[1], M. Ravnikar[1], D. Milkovic[3], I. Mozetic[3], K. Gruden[1]. [1]Department of Biotechnology and Systems biology, National Institute of Biology, Ljubljana, Slovenia, [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland, [3]Department of knowledge technologies, Josef Stefan Institute, Slovenia

PS15-581. **Codon usage pattern of predicted operon-like genes in *Arabidopsis thaliana*.** S. Ikeda[1], M. Wada[1], M. Ooi[1], T. Katsuragi[1], Md. Altaf-Ul-Amin[1], S. Kanaya[1]. [1]Graduate school of Information science, NAIST, Nara, Japan

PS15-582. **Stochastic simulation of metabolic network of *Arabidopsis thaliana* using experimental data.** T. Katsuragi[1], S. Ikeda[1], Md. Altaf-Ul-Amin[1], M. Y. Hirai[2,3], K. Sriyudthsak[2,3], Y. Sawada[2], Y. Yamashita[4], Y. Chiba[4,5], H. Onouchi[3,6], T. Fujiwara[3,8], S. Naito[4,6], F. Shiraishi[7], S. Kanaya[1]. [1]The Graduate School of Information Science, Nara Institute of Science and Technology, Nara, Japan, [2]RIKEN Plant Science Center, Yokohama, Kanagawa 230-0045, Japan, [3]JST, CREST, Kawaguchi, Saitama 332-0012, Japan, [4]Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan, [5]Creative Research Institution, Hokkaido University, Sapporo, 001-0021, Japan, [6]Graduate School of Agriculture, Hokkaido University, Sapporo 060-8584, Japan, [7]Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, Fukuoka 812-8581, Japan, [8]The Faculty of Agriculture, the University of Tokyo, Tokyo 113-8657, Japan

PS15-583. **Development of the micro-particle transportation system using photorepellent response in apo-symbiotic green paramecia.** K. Otsuka[1], T. Kawano[1]. [1]Graduate School of Environmental Engineering, The University of Kitakyushu, Fukuoka, Japan

PS15-584. **Gene discovery of *Colletotrichum acutatum* - strawberry interaction.** D. Amby[1], T. Sundelin[1], M. A. Petersen[2], H. T. Simonsen[1], C. Weitzel[1], B. Jensen[1]. [1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark, [2]Department of Food Science / Quality and Technology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark

Endophytes and parasitic plants

PS18-585. **Infection pattern and growth promotion effects between *Burkholderia cepacia* and**

***Zea mays*.** L.-S. Young[1], H.-Y. Peng[2]. [1]Department of Biotechnology, National Formosa University, Yunlin, Taiwan, [2]Department of Biotechnology, Yuanpei University, Hsinchu, Taiwan

PS18-586. **Rhizophere signal strigolactone produced by plant cell cultures.** T. Nomura[1], K. Yamanaka[1], X. Xie[1], K. Yoneyama[1], T. Kisugi[1], K. Yoneyama[1]. [1]Weed Science Center, Utsunomiya University, Tochigi, Japan

PS18-587. ***PrCYP707A1*, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analog GR24.** P. Delavault[1], M.-M. Lechat[1], J.-B. Pouvreau[1], T. Peron[1], M. Gauthier[1], C. Veronesi[1], G. Montiel[1], Y. Todoroki[2], F. Monteau[3], D. Macherel[4], P. Simier[1], S. Thoiron[1]. [1]Laboratoire de Biologie et Pathologie Vegetales, SFR 4207 QUASAV, LUNAM University, Nantes, France, [2]Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan, [3]LABERCA, Oniris, LUNAM University, Nantes, France, [4]Institut de Recherche en Horticulture et Semences, INRA-Agrocampus Ouest-Universite Angers, SFR 4207 QUASAV, Angers, France

PS18-588. **The phloem network in the parasitic plant *Phelipanche ramosa*; carboxyfluorescein labelling and characterization of three sucrose transporteurs.** T. Peron[1], P. Delavault[2], P. Simier[2]. [1]Agrocampus-Ouest, UMR Institut de Recherche en Horiculture et Semences (INRA, Agorcampus Ouest, Angers University), SFR QUASAV, Angers, France, [2]Laboratoire de Biologie et Pathologie Vegetales, Nantes University, Nantes, France

PS18-589. **Endophytic *Bradyrhizobium* fix nitrogen in sweet potatoes.** J. Terakado-Tonooka[1,2], S. Fujihara[1], Y. Ohwaki[1]. [1]Soil Science and Plant Nutrition division, National Agricultural Research Center, Tsukuba, Japan, [2]JSPS Research Fellow

PS18-590. **Vertical and horizontal transmission of endophyte fungus *Neotyphodium lolii* in perennial ryegrass (*Lolium perenne* L.) plants.** B. Wiewiora[1], G. Zurek[2]. [1]Department of Seed Science and Technology, Plant Breeding and Acclimatization Institute, National Research Institute,Radzikow, Poland, [2]Departament of Grasses, Legumes and Energy Plants, Plant Breeding and Acclimatization Institute, National Research Institute, Radzikow, Poland

PS18-591. **Germination stimulants of *Phelipanche ramosa* in the rhizosphere of *Brassica napus* are derived from the glucosinolate pathway.** B. Auger[1], J.-B. Pouvreau[1], Z. Gaudin[1], K. Pouponneau[2], K. Yoneyama[3], G. Montiel[1], B. Le Bizec[2], K. Yoneyama[3], P. Delavault[1], R. Delourme[4], P. Simier[1]. [1]Laboratoire de Biologie et Pathologie Vegetales, IFR 149 QUASAV, LUNAM Universite, Nantes, France, [2]LUNAM Universite, Oniris, LABERCA, 44307 Nantes, France, [3]Utsunomiya University, Weed Science Center, Utsunomiya 321-8505, Japan, [4]INRA, Agrocampus Ouest, Universite de Rennes 1, UMR1349 IGEPP, 35653 Le Rheu, France

PS18-592. **Transcriptome analysis of the parasitic plant *Phtheirospermum japonicum* indicatesrole of Subtilisin-like proteases in plant parasitism.** J. K. Ishida[1], S. Yoshida[2], E. Wafula[3], C. W. dePamphilis[3], S. Namba[1], K. Shirasu[2]. [1]Graduate School of Agricultural and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan ZIP code 113-8657, [2]Plant Science Center, RIKEN, Yokohama, Japan, [3]Department of Biology, The Pennsylvania State University, USA

PS18-593. **Functional analysis of NADPH oxidases and regulatory factors of fungal endophyte *Epichloë festucae* in cell fusion and conidiation.** Y. Kayano[1], A. Tanaka[1], B. Scott[2], D. Takemoto[1]. [1]Graduate School of Bioagricultural Sciences, University of Nagoya, Aichi, Japan, [2]Institute of Molecular BioSciences, Massey university, Palmerston North, New Zealand

PS18-594. **Draft genome sequences of the parasitic *Striga* species.** S. Yoshida[1], R. Manabe[2], M. P. Timko[3], K. Shirasu[1]. [1]Plant Science Center, RIKEN, Yokohama, Japan, [2]Omics Science Center, RIKEN, [3]Department of Biology, University of Virginia

PS18-595. **Identification of a novel fungal nuclear protein, NsiA, essential for symbiotic infection of endophytic fungus *Epichloë festucae*.** Y. Ozaki[1], F. Akano[1], A. Tanaka[1], S. Sanjay[2], B. Scott[2], D. Takemoto[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, [2]Institute of Molecular BioSciences, Massey university, Palmerston North, New Zealand

Biotechnology

PS19-596. **Effects of sowing density on yield and quantitative characteristics of soybean.** K. Shamsi[1],

S. Kobraee[1]. [1]Department of Crop Production and Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran

PS19-597. **Multiple mechanisms for soil phosphate solubilization: acid and more.** T. Repas[1,2], D. Greenshields[2], S. Kaminskyj[1]. [1]Department of Biology, University of Saskatchewan,, [2]Novozymes BioAg Ltd., 3935 Thatcher Ave., Saskatoon, SK S7R 1A3

PS19-598. **Host-induced gene silencing in fungal pathogens of cereals.** D. Nowara[1], G. Hensel[1], J. Kumlehn[1], P. Schweizer[1]. [1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

PS19-599. **Effect of compost and chemical fertilizer on vegetable growth and microbial community structure in soil.** S. Marsudi[1], Y. Sago[1], S. Yamada[1], Y. Atsuta[1], H. Daimon[1]. [1]Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan

PS19-600. **Engineering plant cell walls for second generation biofuel production.** C. Cook[1], P. G. Bolwell[1], A. Devoto[1]. [1]School of Biological Sciences, Royal Holloway, University of London, Egham, United Kingdom

PS19-601. **Evaluation of IRES-mediated translation efficiency of viral 5' untranslated regions (UTRs) by multi-color luciferase reporter system in higher plants.** R. Ogura[1], C. Hara[2], A. Inamoto[2], K. Nakahama[2], N. Matsuo[2], K. Hiratsuka[2]. [1]Venture Business Laboratory, Yokohama National University, Kanagawa, Japan, [2]Graduate School of Environmental and Information Sciences, Yokohama National University, Kanagawa, Japan

PS19-602. **Detection of quantitative trait loci for partial blast resistance by next-generation whole genome re-sequencing in the rice cultivar Nortai.** H. Takagi[1,2], A. Abe[1], K. Yoshida[3], S. Kosugi[4], H. Yaegashi[1], S. Natsume[1], M. Tamiru[1], L. Cano[3], S. Kamoun[3], R. Terauchi[1]. [1]Iwate Biotechnology Research Center, Iwate, Japan, [2]United Graduate School of Iwate University, Iwate, Japan, [3]The Sainsbury Laboratory, Norwich, UK, [4]Kazusa DNA Research Institute, Chiba, Japan

PS19-603. **Development of endophytic bacterial inoculants possessing growth promotion traits for practical application in bio-energy plant species.** N. A. Otieno[1], J. Culhane[1], K. Germaine[1], D. Brazil[1], D. Ryan[1], D. Dowling[1]. [1]Institute of Technology (IT) Carlow, Carlow, Republic of Ireland

PS19-604. **Homology-independent breakdown of papaya transgenic resistance by super virus strain and the solution.** Y.-J. Kung[1], B.-J. You[1], K.-C. Chen[1], C.-H. Huang[1], H.-J. Bau[1], S.-D. Yeh[1]. [1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

PS19-605. **Development of abaca (*Musa textilis* Nee) putative resistant lines against *Banana bunchy top virus* and *Banana bract mosaic virus* through induced mutation breeding.** T. O. Dizon[1], O. P. Damasco[1], M. S. Pinili[2], I. T. Lobina[1], A. G. Lalusin[1], K. T. Natsuaki[2]. [1]Crop Science Cluster-Institute of Plant Breeding, University of the Philippines Los Banos, Laguna, Philippines, [2]Department of International Agricultural Development, Tokyo University of Agriculture, Tokyo, Japan

PS19-606. **A single chain variable fragment antibody against a *Fusarium virguliforme* toxin for enhancing tolerance of soybean to sudden death syndrome.** H. K. Brar[1], M. K. Bhattacharyya[1]. [1]Agronomy Department, Iowa State University, Ames, Iowa, USA

Genomics and evolution of virulence in pathogenic fungi and oomycetes

PS20-607. **Deep-sequencing of multiple race-leaf rust, *Puccinia triticina* genomes and transcriptomes during wheat infection.** G. Bakkeren[1], D. L. Joly[1], X. Wang[2], N. Thiessen[3], G. Taylor[3], S. D. Jackman[3], I. Birol[3], S. J. M. Jones[3], B. D. McCallum[2], R. C. Hamelin[4], B. J. Saville[5]. [1]Agriculture & Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, [2]Agriculture & Agri-Food Canada, Cereal Research Center, Winnipeg, MB, [3]Michael Smith Genome Sciences Centre, Vancouver, BC, [4]Canadian Forest Service, Laurentian Forestry Centre, Sainte-Foy, QC & Dept. of Forest Sciences, University of British Columbia, Vancouver, BC, [5]Trent University, Forensic Science Program, Peterborough, ON

PS20-608. **An analysis of gene families evolution in Sordariomycetes reveals a high level of gene loss in many phytopathogens.** R. A. Tiburcio[1], L. C. Nascimento[1], M. H. Moraes[1], G. G. Pereira[1], O. G. Cabrera[1]. [1]Instituto de Biologia da Universidade Estadual de Campinas - UNICAMP

PS20-609. **Various stress conditions induce somatic homologous recombination in *Magnaporthe oryzae*.** T. Arazoe[1], S. Ohsato[1], T. Arie[2], K. Yoneyama[1], S. Kuwata[1]. [1]School of Agriculture, Meiji University, Kanagawa, Japan, [2]Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

PS20-610. **Genomic and transcriptomic analysis of two *Colletotrichum* species.** P. Gan[1], Y. Takano[2], R. Manabe[6], R. O'Connell[3], Y. Kubo[5], Y. Narusaka[4], K. Shirasu[1]. [1]RIKEN Plant Science Center, RIKEN Yokohama Institute, Yokohama, Japan, [2]Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [3]Max-Planck-Institute for Plant Breeding Research, Department of Plant-Microbe Interactions, Germany, [4]Research Institute for Biological Sciences, Okayama, Japan, [5]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan, [6]RIKEN Genomic Sciences Center, RIKEN Yokohama Institute, Yokohama

PS20-611. **Genomics, transcriptomics and proteomics analyses of the fungal pathogen *Ceratocystis cacaofunesta* provide new insights on the control of the wilt disease of cacao.** O. G. Cabrera[1], A. B. Ambrosio[1], L. C. Nascimento[2], P. J. P. L. Teixeira[1], B. V. Oliveira[1], D. P. T. Thomazella[1], R. A. Tiburcio[1], M. H. Moraes[1], A. F. P. Leme[2], M. F. Carazzolle[1,3], P. Mieczkowski[4], G. A. G. Pereira[1]. [1]Department of Genetic, Evolution and Bioagents, University of Campinas, Sao Paulo, Brazil, [2]2 Laboratorio Nacional de Biociecias-LNBio, Associacao Brasileira de Tecnologia de Luz Sincrotron, Campinas, SP, Brazil., [3]Centro Nacional de Processamento de Alto Desempenho, Universidade Estadual de Campinas, SP, Brazil., [4]High-Throughput Sequencing Facility, University of North Carolina, Chapel Hill NC, USA.

PS20-612. **Difference in pathogenicity relating genes of *Magnaporhe oryzae* infecting ryegrass and rice based on next-generation genome sequencing.** T. Tsukiboshi[1], T. Kiyoshi[1], I. Okabe[1], A. Masunaka[1]. [1]NARO Institute of Livestock and Grassland

PS20-613. **Comparative genome structure analysis and screening of pathogenicity related genes of *Magnaporthe* isolates by SOLiD whole genome resequencing.** S. Urushizaki[1], Y. Kobayashi[1], I. Kobayashi[1]. [1]Grad. Schl. of Region. Innova. Studies/ Life Sci. Res. Cntr, Mie Univ.

PS20-614. **Structure of a conditionally dispensable pathogenicity chromosome of the tomato pathotype of *Alternaria alternata*.** Y. Akagi[1], T. Tsuge[2], M. Kodama[1,3]. [1]Faculty of Agriculture, University of Tottori, Japan, [2]Graduate school of bioagricultural sciences, University of Nagoya, Japan, [3]Fungus/mushroom resource and research center

PS20-615. **The *LaeA*-like methyltransferase gene (*AaLAE*) regulates host-specific toxins production and pathogenicity in the fungal plant pathogen *Alternaria alternata*.** K. Takao[1], Y. Akagi[1], Y. Harimoto[2], T. Tsuge[2], M. Kodama[1]. [1]Faculty of Agriculture, University of Tottori, Japan, [2]Graduate School of Bioagricultual Sciences, University of Nagoya, Japan

PS20-616. **Genetic change of *Pyricularia grisea* in different host genome.** U. W. Suharsono[2], S. Listiyowati[2], G. Rahayu[2], A. Hartana[2]. [1]Department of Biology, Bogor Agricultural University, Bogor, INDONESIA, [2]Bogor Agricultural University

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Molecular Biosciences, Institute for Molecular Bioscience, and Centre for Infectious Disease Research, University of Queensland, Brisbane 4072, Australia, [2]School of Biological Sciences, Flinders University, G.P.O. Box 2100, Adelaide 5001, Australia, [3]CSIRO Plant Industry, Canberra, Australia

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PS21-621. **Crystal structure of a flax cytokinin oxidase and interaction studies with a fungal effector.** L. Wan[1], M. Koeck[2], S. Williams[1], X. Zhang[1], J. Ellis[2], P. Dodds[2], B. Kobe[1]. [1]School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience, and Centre for Infectious Disease Research, University of Queensland, Brisbane 4072, Australia, [2]CSIRO Plant Industry, Canberra, Australia

PS21-622. **The GTP-form structure of small GTPase OsRac1, a key player in rice innate immunity.** K.-I. Kosami[1], I. Ohki[2], K. Hayashi[2], R. Tabata[2], S. Usugi[2], T. Kawasaki[2,3], A. Nakagawa[1], T. Fujiwara[1], K. Shimamoto[2], C. Kojima[1]. [1]Inst. Prot. Res., Osaka Univ, Osaka, Japan, [2]2Grad. Sch. Nat. Sci, Nara, Japan, [3]Sch. of Agri, Kinki Univ, Nara, Japan

ABSTRACTS

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Abstract of Opening Lecture

OL-1

Innate immunity in mammals

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The innate immune system is an evolutionally conserved host defense mechanism against pathogens. Innate immune responses are initiated by pattern recognition receptors (PRRs), which recognize specific structures of microorganisms. Among them, Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria to fungi, protozoa and viruses, and play a major role in innate immunity. Individual TLRs recognize different microbial components, and give rise to different patterns in gene expression. We are now focusing on the role of genes induced in response to TLR stimulation, particularly the genes that are rapidly induced in a MyD88-dependent manner within 30 min after LPS stimulation. Among them, we have recently identified a novel gene named Zc3h12a which has a CCCH-type zinc finger domain. The knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. Subsequent studies showed that Zc3h12a is a nuclease involved in destabilization of IL-6 and IL-12mRNA. We renamed it Regulatory RNase-1 (Regnase-1) based on the function. We recently found that the IKK complex controls IL-6m stability by phosphorylating Regnase-1 in response to IL-1R/TLR stimulation. Phosphorylated Regnase-1 underwent ubiquitination and degradation. Taken together, Regnase-1 is involved not only in the late phase suppression of TLR-mediated IL-6 mRNA expression but also in the gbrake h on the initial IL-6mRNA induction.

Abstract of Award Lecture

AL-1

Innate immunity effectors and virulence factors in symbiosis

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In symbiosis, host cells coexist with a multitude of bacteria and usually share metabolites, thus providing each other with missing vital components. *Rhizobium*-legume symbiosis results in the formation of root nodules where intracellular bacteria reduce atmospheric nitrogen and supply ammonia for plant growth in exchange for energy and C sources from the plant. This bacterium-plant interaction used to be considered mutually beneficial. This view has, however, changed drastically upon the discovery that certain plants exploit their bacterium partners by directing them into an irreversible, terminal differentiation with no chance to return to the free-living state. The mechanism of plant dominance has been elucidated in *Medicago truncatula* where >600 antimicrobial peptides, related to the effectors of innate immunity, have adapted and evolved for symbiosis. These peptides are produced in the symbiotic cells and are targeted to the bacteria provoking genome amplification, extreme cell elongation, increased membrane permeability and loss of cell division capacity. The combined action of the peptides keeps the bacteria viable but uncultivable and necessitates the function of the bacterial BacA protein, which is also essential for the establishment of chronic intracellular infection by intracellular mammalian pathogens. *Rhizobium*-legume symbiosis also appears to be a paradigm for other host-bacterium interactions and may help to understand the persistence of intracellular bacteria in eukaryotic cells.

ABSTRACTS

Plenary Lectures

PL1-1

Regulation of surface immune receptor complex activity

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The first layer of plant innate immunity relies on the recognition of microbes via the perception of pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs) by surface localized receptors called pattern recognition receptors (PRRs). In the plant model *Arabidopsis thaliana*, the leucine-rich repeat RKs (LRR-RKs) FLS2 and EFR are the PRRs for bacterial flagellin (or flg22) and elongation factor Tu (or elf18), respectively. Within seconds of PAMP binding, FLS2 and EFR form a ligand-induced complex with the regulatory LRR-RK SERK3/BAK1 leading to phosphorylation of both proteins. Additional SERKs, such as SERK4/BKK1, are recruited in a ligand-dependent manner into EFR and FLS2 protein complexes with different preferences. FLS2 (and potentially EFR) also forms a constitutive complex with the membrane-associated cytoplasmic kinase BIK1 that get phosphorylated in a BAK1-dependent manner upon PAMP binding. BIK1 is a positive regulator of most FLS2- and EFR-mediated responses. Downstream of FLS2 and EFR complexes, activation leads to several rapid responses, including bursts of Ca2+ and reactive oxygen species (ROS), activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), and transcriptional reprogramming, ultimately leading to PAMP-triggered immunity. The mechanisms controlling PRR activation at the plasma membrane and regulating intracellular signalling remain however largely unknown. Using a combination of biochemical and genetic approaches, we have uncovered various components controlling directly the activity of the FLS2 and EFR complexes at the plasma membrane. In addition to underlying the activation of PAMP-triggered immunity, these mechanisms also limit the over-activation of immune responses that would be otherwise detrimental to the plant.

PL1-2

The role of Fusarium effectors in NLR-mediated innate immunity

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The interaction between the fungus *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) and tomato follows a gene-for-gene relationship. Tomato resistance genes *I, I-2* and *I-3* confer resistance to *Fol* based on recognition of Avr1, Avr2 and Avr3, respectively. These three Avrs have been identified among the fungal proteins (Six proteins) found in the xylem sap of Fol-infected tomato plants. Of the R genes, only *I-2* has been cloned; the gene is expressed in xylem contact cells and encodes an intracellular NB-LRR protein. All Fol races have *AVR2* and its expression is induced upon root contact and during colonization of xylem vessels. *AVR2* is a virulence factor as its deletion compromises pathogenicity. *Fol* deploys two strategies to overcome *I-2*-mediated resistance. Race 3 strains carry point mutations in *AVR2* that do not affect virulence but allow it to evade recognition. *AVR1* does not contribute to virulence on susceptible plants but suppresses *I-2* function. Surprisingly, a *SIX5* knockout, like the *AVR2* knockout, becomes virulent on an *I-2* tomato line. Nevertheless, *AVR2* alone is sufficient to induce *I-2*-mediated cell death in *N. benthamina* and in tomato, suggesting that cell death and resistance are not strictly linked. Support for a functional interaction of this effector pair is that their expression is driven by same promoter and that both proteins interact in a Y2H system. We aim to further unravel the molecular mechanisms underpinning the observed virulence and avirulence functions of these effectors and the relevance of the formation of a putative heteromeric complex.

PL1-3

Defensome in rice innate immunity

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We have been studying molecular signaling in rice innate immunity by studying the small GTPase OsRac1 and its interacting proteins by using a variety of methods. We have identified a number of OsRac1-interacting proteins and studied their functions and interactions with other proteins. We found that OsRac1 interacts with two types of receptors; membrane-bound receptor-like kinases and NB-LRR type receptors. OsRac1 forms a protein network with several cheperones and co-chaperones, SGT1, RAR1, Hsp90, Hsp70, and Hop/Sti1. A scaffloding protein, RACK1, also interacts with OsRac1. The OsRac1 network includes enzymes such as NADPH oxidase and CCR which are important for immune responses. Based on genetic, protein-protein interaction, and biochemical studies we propose that OsRac1 is a hub of rice innate immunity where PTI and ETI pathways merge. We also propose that these proteins form complex termed defensome. Based on the recent biochemical analysis we found PTI and ETI receptors form separate defensomes but contain the same chaperones in each defensome. Our results suggest that the defensome complex is a key regulatory unit for rice innate immunity.

PL2-1

Defining the core *Arabidopsis thaliana* root microbiome

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Land plants associate with a root microbiota distinct from the complex microbial community present in surrounding soil. The microbiota colonizing the rhizosphere (immediately surrounding the root), and the endophytic compartment (within the root), contribute to plant growth, productivity, carbon sequestration, and phytoremediation. Colonization of the root occurs despite a sophisticated plant immune system, suggesting finely-tuned discrimination of mutualists and commensals from pathogens. Genetic principles governing the derivation of host-specific endophyte communities from soil communities are poorly understood. We pyrosequenced the bacterial 16S rRNA gene of >600 *Arabidopsis thaliana* plants to test the hypotheses that the root rhizosphere and endophyte compartment microbiota of plants grown under controlled conditions in natural soils are (i) sufficiently dependent on the host to remain consistent across different soil types and developmental stages, and (ii) sufficiently dependent on host genotype to vary between inbred Arabidopsis accessions. We describe different bacterial communities in two geochemically distinct bulk soils, and in rhizosphere and endophyte compartments prepared from roots grown in these soils. The communities in each compartment are strongly influenced by soil type. Endophyte compartments from either soil feature overlapping low-complexity communities that are markedly enriched for Actinobacteria and specific families from other phyla, notably Proteobacteria. Some bacteria vary quantitatively between plants of different developmental stages and genotypes. Our work provides unprecedented rigor to define an endophyte compartment microbiome, facilitating controlled dissection of plant-microbe interactions derived from complex soil communities.

PL2-2

Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota

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The plant root defines the interface between a multicellular eukaryote and soil, one of the richest microbial ecosystems on earth. Remarkably, soil bacteria are able to multiply inside roots as benign endophytes and modulate plant growth and development, with implications ranging from enhanced crop productivity to phytoremediation. We describe methodology to characterize and compare soil and root-inhabiting bacterial communities, which reveals not only a function for metabolically active plant cells but also for inert cell wall features in the selection of soil bacteria for host colonization. We show that roots of *Arabidopsis thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetesand Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. The identification of soil type-specific members within the root-inhabiting assemblies supports our conclusion that these represent soil-derived root endophytes. Surprisingly, plant cell wall features of other tested plant species appear to provide a sufficient cue for the assembly of ~30% of the Arabidopsis bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. Thus, this root sub-community may not be Arabidopsis-specific but saprophytic bacteria that would naturally be found on any plant root or plant debris in the tested soils. In contrast, colonization of Arabidopsis roots by members of the Actinobacteria depends on additional cues from metabolically active host cells.

PL2-3

Oomycetes, effectors, and all that jazz

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The field of plant-microbe interactions has now coalesced around a general model. The major classes of molecular players both from plants (surface and intracellular immune receptors) and microbes (PAMPs and effectors) have now been revealed. This model applies to plant pathogenic oomycetes, such as the Irish potato famine organism Phytophthora infestans. These pathogens secrete a diverse repertoire of effector proteins that modulate host innate immunity and enable parasitic infection. Some effectors are targeted to the apoplast (apoplastic effectors), while others, notably the RXLR and CRN families, are translocated inside the host cell (cytoplasmic effectors). A number of RXLR effectors activate immunity in plants that carry cognate R immune receptors of the NBS-LRR class. Other oomycete molecules, such as elicitins, have features of PAMPs; they activate immunity via surface receptors and their modulators, which include the receptor-like kinase BAK1/SERK3. We study several aspects of oomycete-plant interactions with a focus on two questions: (i) how do effectors evolve, how do they adapt to their host targets and evade recognition by immune receptors?; (ii) how do effectors function, how exactly do they modulate host immunity? This presentation will highlight recent advances on these topics. We made important progress with, notably, the elucidation of the 3D structures of RXLR effectors, novel insights into how effectors modulate host cell immunity, and the discovery that some RXLR effectors accumulate around haustoria to interfere with the execution of polarized host defenses. Finally, we are also concerned with exploiting basic knowledge on effector biology to impact agriculture.

PL2-4

Systems biology initiatives for the rice blast fungus

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The research goal of my laboratory is to elucidate the molecular mechanisms of fungal pathogenesis and interactions between rice blast pathogen, *Magnaporthe oryzae* and its host plant, rice at the genomic level. Rice blast is a compelling model system for studying host parasite interactions due to its socioeconomic impact and the availability of both the rice and fungal genomic sequences. In an attempt to understand the molecular mechanisms of rice blast, we have been taking both forward and reverse genetics approaches. Our researches using reverse genetics approach focus on identifying and characterizing the genes involved in signal transduction pathways leading to appressorium formation, genes encoding transcription factors, and genes that are required for post penetration stages. For forward genetics studies, we carried out a large scale insertional mutagenesis of the *M. oryzae* strain KJ201 via *Agrobacterium tumefaciens* mediated transformation, generating over 25,000 mutants. We also developed high throughput phenotype screening system that enables rapid and robust assay of mutant phenotypes. Those mutants are stored and maintained in the

Center for Fungal Genetic Resources. In addition to our endeavor to functional genomics, we built a cyber infrastructure for storage of heterogeneous data and analysis of such data in multiple contexts. The genome sequence information of *M. oryzae* as well as most of the results from experimental biology is housed in our customized databases. Our comprehensive and integrative approaches coupled with a web based Laboratory Information Management System would provide a novel platform for systems biology initiatives for fungal pathogenesis.

PL3-1

Partitioning of effector-triggered immune outputs in plant cells

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Plants have evolved a multi-layered innate immune system to defend themselves against microbial pathogens. Recognition of pathogen effectors in their attempts to disable basal cellular immunity is governed by host intracellular NB-LRR (Nucleotide binding/Leucine Rich Repeat) receptors which then activate defense and cell death pathways. NB-LRR triggered defenses need to be tightly controlled because they are energetically costly and disturb normal metabolism and growth. We have been studying the molecular interactions, protein structural features and subcellular functions of Arabidopsis biotic stress regulator EDS1 (Enhanced Disease Susceptibility1) as a means to understand plant disease resistance signaling dynamics. EDS1 is a nucleocytoplasmic lipase-like protein which, together with its signaling partners PAD4 and SAG101, controls basal immunity to virulent pathogens and is recruited by intracellular TIR (Toll-Interleukin1-Receptor)-NB-LRR receptors for effector-triggered immunity. I'll describe our recent data on resistance mediated by Arabidopsis TIR-NB-LRR receptor RPS4 in response to the *Pseudomonas syringae* Type III secreted effector, AvrRps4. Using this recognition-response system we have identified RPS4-EDS1 defense branches operating in different parts of the cell and processes inside nuclei associated with transcriptional defense amplification. We propose a model in which resistance outputs are coordinated across cell compartments, allowing the plant to respond flexibly to a particular mode or site of pathogen effector interference.

PL3-2

How oomycete pathogens of Arabidopsis cause or fail to cause disease

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Plant disease resistance mechanisms are initiated by surface receptors and cytoplasmic receptors that respectively recognize conserved or variable pathogen components. To suppress defence, pathogens deliver effector molecules into host cells. Understanding these effectors is important to identify new probes to host defence mechanisms and develop durable resistance strategies. Although the effector complements of bacteria are becoming well defined, and the mechanisms of many bacterial effectors are quite well understood, the effectors of the fungal and oomycete pathogens that cause the most serious crop losses are still poorly characterized. Recent advances in sequencing methods now enable us to define genomes of such pathogens and to predict gene models. As a model system, we work with the downy mildew pathogen *Hyaloperonospora arabidopsidis* (Hpa) and two other oomycete pathogens, *Albugo laibachii* and *A. candida*. The Hpa genome is available. We used Illumina paired read sequencing to assemble sequences of multiple races of *Albugo laibachii*, a pathogen that is particularly effective at shutting down host defence, and also of multiple *A. candida* races. We are using association genomics to correlate genetic variation in the secretome of *Albugo laibachii* with virulence or avirulence on specific Arabidopsis accessions. In addition, we are using the MAGIC inbred lines of Kover and Mott, to reveal transgressive segregation for susceptibility to Brassica-infecting *A. candida* strains, in order to identify genes for non-host resistance. An update on recent progress will be presented.

PL3-3

Messages from powdery mildew DNA: how interplay with the host moulds the pathogen genomes

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The genomes of obligate biotrophic fungi that have become completely dependent on a plant to survive and multiply have common hall-marks. These features indicate a striking instance of convergent evolution in these eukaryotic microbes. In this talk I will illustrate using the example of the barley powdery mildew *Blumeria graminis*, how a compulsory addiction to a biotrophic lifestyle has lead to reductions of common genes and gene families on the one hand, and to an extraordinary expansion of lineage-specific genes that we postulate encode effectors devoted to controlling host immunity and defence. I will present the analysis of recent data to support this hypothesis and discuss how life-style and "choices" in reproductive strategies appear to have driven the manner in which these organisms have evolved.

PL4-1

Reprogramming root cells for arbuscular mycorrhizal (AM) symbiosis

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In natural ecosystems, most vascular flowering plants live in symbiosis with arbuscular mycorrhizal (AM) fungi. These mutually beneficial associations develop in the roots, where the fungus colonizes the cortex to obtain carbon from the plant. In addition to inhabiting the root, the fungus establishes hyphal networks in the soil, via which phosphorus and other mineral nutrients are transferred to the root. Thus, the symbiosis has a beneficial impact on plant health. In AM symbiosis, nutrient exchange occurs between branched hyphae, called arbuscules and the plant cortical cell in which they reside. Arbuscule development is a complex process that requires not only the differentiation of the fungus, but also major alterations to the colonized cortical cell, including the deposition of the periarbuscular membrane around the arbuscule. We are interested in the molecular and cellular events that underlie development of arbuscules and the trafficking of proteins to the periarbuscular membrane. We have identified three *Medicago truncatula* genes, Vapyin, STR and STR2, that are required for arbuscule formation and a phosphate transporter, MtPT4, that is required for symbiotic Pi transport. The roles of these genes and the trafficking of MtPT4 to the periarbuscular membrane will be discussed.

PL4-2

Evolution of Rhizobium nodule symbiosis

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Both rhizobia and AM fungi can establish an endosymbiosis with plants. In both cases the host membrane forms compartments that act as a symbiotic interface to control exchange of compounds; these compartments are at the heart of endosymbiosis. At first glance both symbioses seem rather different. The AM fungal symbiosis is ancient, as it evolved 450 million years ago, roughly the same time as land plants. About 80% of today's land plant species maintained this ancient symbiosis, underlining its ecological importance. In contrast, nitrogen fixing rhizobium symbiosis is specific for legumes (*Fabaceae*), with the important exception of the genus *Parasponia* in the *Cannabaceae* (cannabis family). The rhizobium-legume symbiosis is as old as the legume family (˜60 million years), whereas rhizobium-Parasponia symbiosis evolved even more recent (<10million years). Despite these differences recent research has revealed striking similarities. AM fungi and rhizobium secrete similar lipochito-oligosaccharides (LCOs, Nod and Myc factors) and in the non-legume Parasponia, the mycorrhizal and rhizobial LCOs are even recognized by the same "Nod factor" receptor (Op den Camp et al., Science, 2011). In legumes Nod factor receptors are not essential for the interaction with AM fungi which suggests that "Nod factor" receptor genes duplicated and diverged by neo-functionalization. Previous studies had already revealed that a common signalling pathway was activated by rhizobia and AM fungi. In addition to these similarities in signalling, we showed that the cellular machineries involved in the formation of the symbiotic interfaces involves the same exocytotic pathway (Ivanov et al., PNAS, 2012, in press).

PL4-3

What did we learn from the MOSes?

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Plant nucleotide-binding leucine-rich repeat (NB-LRR) proteins serve as intracellular immune sensors to detect pathogen effectors and trigger immune responses. The Arabidopsis *snc1* mutant carries a gain-of-function mutation in a gene encoding a TIR-NB-LRR protein, resulting in the constitutive activation of plant defense responses. *snc1* suppressor screens undertaken using fast neutron, EMS or T-DNA insertional mutagenesis, resulted in the identification of a large number of *modifier of snc1* (*mos*) mutants, which either completely or partially suppresses the autoimmune phenotypes of *snc1*. Previous studies on the mos mutants revealed that nucleocytoplasmic trafficking and protein modifications are critical for the regulation of NB-LRR protein-triggered immunity. Our more recent findings suggest that alternative splicing and the regulation of NB-LRR gene expression levels by histone modification also play important roles in the regulation of NB-LRR protein-mediated defense.

PL4-4

Chitin receptors in plant immunity

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Chitin is a major component of fungal cell walls and serves as a molecular pattern for the detection of these microbes. Various plants are equipped with a sensitive system to detect chitin and initiate defense responses. We previously identified two types of cell surface receptors, CEBiP and CERK1/OsCERK1, involved in the perception of chitin in rice and Arabidopsis (1-3). CEBiP, a GPI-anchored protein that binds chitin oligosaccharides specifically, forms a heteromeric receptor complex with a receptor-like kinase OsCERK1 ligand dependently (3). This seems to trigger downstream signaling leading to defense responses. On the other hand, we recently found that Arabidopsis chitin receptor does not require CEBiP-like molecules for chitin signaling, though a CEBiP homologue in Arabidopsis is biochemically very similar to rice CEBiP.Importance of chitin recognition in plant immunity has been supported not only by the infection experiments with KO mutants of these receptors but also by recent findings on the fungal effectors that inhibit the perception of chitin oligosaccharides by these receptors (4-5). Interestingly, lipochitin oligosaccharides secreted by nodulating rhizobia and mycorhizal fungi serve as symbiotic signals for host plants. These molecules are also recognized by the receptors structurally related to CERK1. We recently showed that a very limited mutation in the kinase domain of CERK1 could switch cellular responses from defense to symbiosis, indicating close evolutional relationships between these systems (6).
(1) Kaku et al., 2006; (2) Miya et al., 2007; (3) Shimizu et al., 2010; (4) de Jonge et al., 2010; (5) Mentlak et al., 2012; (6) Nakagawa et al., 2011.

PL5-1

Recognition of rust effectors in plant innate immunity

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Rust fungi cause economically important diseases of cereal crops worldwide. We have been studying how the plant immune system can recognise and respond to these pathogens in order to develop novel disease control strategies. Rusts are obligate parasites of plants, and have evolved an intimate cellular association with their hosts. They produce a specialised infection structure called the haustorium which directly penetrates an infected cell and is the main site of nutrient extraction for the fungus. A suite of disease effector proteins are secreted from haustoria and enter the host cells where they may allow the rust to commandeer host cell biology. It is these translocated effector proteins that are recognised by host immune receptors, known as resistance (R) proteins. We are exploring the structure and function of host-translocated effectors, their recognition by host immune receptors, and the receptor signalling activation process, which offers the opportunity to experimentally engineer new recognition capacities.

PL5-2

Signaling networks in plant innate immunity

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Microbe-associated molecular patterns (MAMPs) are perceived by cell-surface receptors to mount pattern-triggered immunity (PTI) for broad-spectrum microbial resistance in plants. However, successful pathogens acquired virulence effectors to suppress PTI. To confine or eliminate pathogens, plants further evolved polymorphic R proteins to directly or indirectly recognize effectors and initiate effector-trigger immunity (ETI) accompanied with localized PCD and transcriptional reprogramming. How distinct cell-surface and intracellular immune sensors trigger overlapping or/and differential primary immune signaling responses are still largely open questions. Chemical genetic analyses and genome-wide gene expression profiling reveal that complex MAPK cascades and CDPK activation mediate convergent signaling triggered by diverse MAMPs. Our recent studies discover the surprisingly central roles of CDPK but not MAPK activation in primary and cell-autonomous ETI signaling. Consistent with the activation of specific CDPKs by MAMPs and effectors, some CDPK-specific marker genes are activated by both signaling pathways. However, MAMPs trigger a transient Ca2+ increase and CDPK activation, whereas Ca2+ increase induced by effectors lasts for hours accompanied with sustained CDPK activation, which is responsible for bifurcate transcriptional reprogramming and PCD. Thus, the timing, amplitude and duration of differential CDPK activation appear to dictate their substrate specificity and differential transcriptional reprogramming in ETI and PTI signaling. The current data imply that activation of distinct cell-surface receptor kinases recognizing different MAMPs and intracellular NLR (NB-LRR) immune sensors sensing diverse pathogen-encoded effectors initiate differential primary signaling events, which trigger both overlapping and specific immune responses to maximize plant defense to pathogen attacks.

PL5-3

Molecular basis of ATR1 effector recognition and activation of the RPP1 NLR innate immune receptor complex

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The Arabidopsis thaliana RPP1 disease resistance protein specifically recognizes its cognate ATR1 effector protein of *Hyaloperonospora arabidopsidis* to activate disease resistance. This system provides a unique opportunity to exploit 3-D structural information of the solved crystal structure of ATR1 and the 3-D molecular modeling of the LRR domain of RPP1 to define surface exposed amino acid residues that define recognitional interfaces that are important for binding and the release of the negative regulation and activation of the RPP1 protein. The solving of the 3-D crystal structure of the ATR1 effector protein has revealed an unprecedented, two-domain, dimeric fold in this protein. We have identified conserved hydrophobic surface residues that can drive the design of targeted and random PCR mutagenesis experiments to discover the functional regions of this protein involved. Furthermore, we have demonstrated that the ATR1 protein associates in planta with the LRR domain of the cognate RPP1 protein suggesting that these two proteins directly interact in planta. These observations are further supported by the isolation and characterization of RPP1 gain of function mutants that can now recognize previously unrecognized alleles of ATR1. These studies exemplify the power of combining structural and biological approaches to reveal critical domains involved in pathogen effector recognition. A recent update on our progress to understand the preactivation and postactivation state of the RPP1 resistance protein complex will be presented.

PL6-1

Biochemical functions of bacterial effectors and plant immunity

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We have been using *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *campestris* type III effectors as models to understand how pathogens modulate host processes to their advantage and how this has led to the evolution of effector-triggered immunity in plants. Our results show that type III effectors often use novel biochemical mechanisms to attack plant immune proteins. For example, we recently found that the *Xanthomonas campestris* effector AvrAC inhibits plant immunity and contributes to Xcc virulence in Arabidopsis by targeting BIK1 and RIPK, two receptor-like cytoplasmic kinases mediating immune signaling. AvrAC is a new enzyme that uridylylates the conserved serine and threonine residues in the activation loop of BIK1 and RIPK. The uridylylation on these residues masks the phosphorylation sites, inhibits the kinase activity, and blocks downstream signaling. Together with previous work, it becomes clear that immune signaling modules including the immune receptor kinase complexes, receptor-like cytoplasmic kinases, and MAPK cascades are targeted frequently by multiple type III effectors, suggesting that these modules are major hubs in plant innate immunity. Furthermore, these studies have uncovered striking parallels between the biochemical mechanism for effector virulence function and the mechanism by which effectors are recognized in by plant disease resistance proteins, providing insight into plant immune signaling network and host-pathogen co-evolution. Ongoing work on type III effectors and plant immunity will be presented.

PL6-2

Targeting transcription factors: mechanism of effector repression

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Manipulation of host protein sumoylation by pathogens is an important virulence strategy to suppress immunity. The direct link between protein sumoylation and eukaryotic transcription suggests that pathogens might directly modulate the sumoylation state of transcription factors. Here we provide evidence that XopD, a SUMO protease from *Xanthomonas campestris* pathovar *vesicatoria* (Xcv), directly interferes with plant transcription to modulate ethylene (ET) responses during infection. XopD is required to promote Xcv growth in tomato leaves and to suppress disease symptom development. Given that XopD contains two EAR motifs implicated in ET signaling and transcription repression, we hypothesized that XopD may directly regulate ET production and/or signaling. Consistent with this hypothesis, ET gas and biosynthesis mRNAs were significantly higher in Xcv delta xopD-infected leaves compared to Xcv-infected leaves. Both ET production and perception were required for tomato immunity and symptom development. Inspection of tomato ERFs expressed in Xcv-infected leaves suggested that SlERF4 is a putative XopD substrate. Virus-induced gene silencing in tomato revealed that SlERF4 mRNA expression was required for Xcv delta xopD-induced ET production and ET-stimulated immunity. XopD was found to colocalize with SlERF4 in subnuclear foci and hydrolyze tomato SUMO1 from K53 of SlERF4 resulting in SlERF4 destabilization. Mutation of K53 to R53 prevented SlERF4 sumoylation, decreased SlERF4 levels, and reduced SlERF4-dependent transcription. We conclude that XopD directly binds and desumoylates SlERF4 to repress ET induced-transcription required for Xcv immunity. This is the first example of a pathogen SUMO protease that targets a host sumoylated transcription factor to suppress defense.

PL6-3

Signal transduction in plant root symbiosis

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We are interested in unraveling the molecular mechanisms involved in the intracellular accommodation of symbiotic microorganisms by plants. Legumes form symbiosis with phosphate-acquiring arbuscular mycorrhiza fungi and nitrogen-fixing rhizobia. Forward genetics has identified a series of plant genes required for early developmental stages of both symbioses. The predicted protein products of these common symbiosis genes include a receptor-like kinase, nuclear localized ion channels and components of the NUP84 sub-complex of the nuclear pore. These components act upstream of symbiosis-induced calcium spiking, which is likely to be decoded by a complex formed by a calcium and calmodulin-dependent protein kinase and CYCLOPS, a nuclear protein with a coiled-coil domain. Recent progress in analyzing the function of individual symbiosis signaling components at the mechanistic level will be presented.

PL6-4

Establishing beneficial interactions with the symbiosis signalling pathway

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The establishment of rhizobial and mycorrhizal symbioses requires the common symbiotic signalling pathway that utilises oscillations in calcium as a secondary messenger. Despite commonalities in signalling, it is clear that differential outputs occur from the signalling pathway, which coordinate specific aspects of each symbiosis. Calcium oscillations are perceived by a calcium and calmodulin dependent protein kinase (CCaMK) and gain-of-function mutations in this protein autoactivate both nodulation and mycorrhizal responses. Downstream of CCaMK are a suite of GRAS-domain transcription factors, with NSP2 having dual roles in nodulation and mycorrhization, but NSP1 and RAM1 functioning specifically in nodulation or mycorrhization respectively. NSP2 interacts with both NSP1 and RAM1 and this suggests that the specificity of symbiosis signalling may be defined by the specific formation of one or the other transcription factor complex. NSP1 can bind the promoters of Nod factor inducible genes and of particular importance is the activation of the NIN and ERN1 transcription factors. These are necessary for activation of nodulation and bacterial infection. In contrast, RAM1 binds the promoters of mycorrhizal induced genes, including RAM2 a protein that functions in the promotion of mycorrhizal colonisation. While we now have a grasp on the nature of specific downstream responses, the precise mechanisms that ensure the appropriate activation of mycorrhizal or rhizobial-specific responses remains unclear.

PL7-1

Pathogen effector proteins and pathogenicity on plants

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Bacteria serve as useful models for the study of plant-pathogen interactions because they represent a simplified examplar of the antagonistic relationships between host and pathogen. Contributions from many labs across a range of pathosystems have demonstrated a general model for biotrophic interactions, in which invading microbes first stimulate host defences through activation of surface pattern recognition receptors (PRRs), then dampen the host response through delivery of toxins and effectors. A particular interest in my laboratory has been study of recognition of the unrelated Pseudomonas syringae effectors AvrPto and AvrPtoB, which are delivered to the host cell cytoplasm to target PRRs, but can be recognised by the Prf recognition complex of tomato. Prf encodes a nucleotide-binding leucine-rich repeats (NB-LRR) protein that forms a constitutive complex with Pto kinase. By characterising the Prf complex, we have found that it is a sophisticated molecular trap for effectors that target protein kinases: by perturbing one kinase molecule in the multimeric complex, another is activated, thus inducing the defense cascade. Recently, we have broadened our studies to include the obligate biotrophic pathogen wheat stripe rust, which causes devastating crop losses worldwide. As a haustorial pathogen, it is possible to purify the pathogenic niche intact from the plant. We have sequenced the stripe rust genome, and analysed the transcriptomes of different developmental stages of the fungus. We have identified almost a thousand effector gene candidates, and gained considerable insight to pathogenic strategies of the fungus.

PL7-2

Effectors in smut fungi and how they affect virulence

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The fungus *Ustilago maydis* is a biotrophic plant pathogen infecting maize. The most prominent symptoms are large plant tumors in which the fungus proliferates. During host colonization *U. maydis* establishes an extended interaction zone in which fungal hyphae are completely encased by the host plasma membrane. Interaction with the plant is largely determined by protein effectors that are conventionally secreted and exert their function either in the interaction zone or are taken up by host cells and reprogram host responses. Many of these effectors are novel, exist only in related smut fungi and locate to clusters in the genome. In my presentation I will concentrate on transferred effectors, their site of action and function after uptake. In addition I will describe how the transferred chorismate mutase Cmu1 can be used to assay translocation and present evidence that unconventionally secreted effectors also contribute to virulence.

PL7-3

Bacterial manipulation of jasmonate receptor and immunity in plants

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We have been studying how *Pseudomonas syringae* pv. *tomato* (Pst) strain DC3000 causes disease in *Arabidopsis thaliana*. During infection, Pst DC3000 produces a battery of virulence factors to engage multiple host cell types and diverse host physical and chemical barriers. The bacterial type III secretion system (T3SS) delivers effector proteins directly into the host cell, whereas the phytotoxin coronatine mimics the active form of plant hormone jasmonate. Study of the molecular action of T3SS effectors and coronatine has begun to show the great utility of bacterial pathogenesis as a probe in the discovery of new components of the plant immune system, as well as fundamental cellular mechanisms in plants. In this talk, I will discuss our recent research that contributed to the identification of the jasmonate receptor complex and an understanding of the mechanism by which coronatine suppresses host defenses. Our work on T3SS effectors, particularly two functional redundant effectors HopM1 and AvrE, begins to yield insight into an aspect of pathogenesis that may be conserved for bacterial pathogenesis in plants. We have characterized a host target (MIN7) of HopM1 and found MIN7 to be important for PAMP-triggered immunity (PTI), effector-triggered immunity (ETI) and salicylic acid (SA)-dependent resistance. Belonging to the ARF family of guanine nucleotide exchange factors, MIN7 likely contributes to defense-associated intracellular vesicle trafficking for transporting components of PTI, ETI and SA-dependent immunity. Finally, although research on AvrE has been a major challenge to us and other colleagues, progress has been made.

PL8-1

RNA-seq identifies a novel *Xanthomonas* specific plant resistance gene in pepper

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Transcription-activator like effector (TALE) proteins of the plant pathogenic bacterium *Xanthomonas* bind to and transcriptionally activate host susceptibility genes to promote disease. Plants can take advantage of this mechanism, as exemplified by the pepper *Bs3* and rice *Xa27* resistance (*R*) genes that both contain TALE binding sites, which direct transcriptional activation of these *R* genes thereby triggering a defense response. Since mono- and dicot plants evolved the same mechanism to detect *Xanthomonas* pathogens we postulated that transcriptome profiling, instead of the laborious positional cloning approach, could be employed to clone TALE-specific *R* genes. In a proof-of-principle experiment RNA-seq studies identified a candidate for the pepper *Bs4C* gene that mediates recognition of the *Xanthomonas* TALE protein AvrBs4. Genetic mapping and complementation studies indeed confirmed that the candidate transcript corresponds to the pepper *Bs4C* gene. These findings demonstrate that TALE-specific *R* genes can be cloned even from large-genome crop species by a highly-efficient RNA-seq approach.

PL8-2

The role of LysM type receptors in Nod factor perception

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Formation of root nodules in legumes relies on a genetic program controlling and synchronising two processes running in parallel. Nodule primordia are formed from root cortical cells initiating cell divisions and simultaneously a bacterial infection process targets the primordia developing from the cell division foci. Plant receptors involved in perception of bacterial signal molecules are required for triggering signal transduction through these pathways and they are also involved in the specific recognition of rhizobia. The role of Lotus japonicus LysM type serine/threonine receptor kinases in perception of Nod-factor signals from bacterial microsymbionts during nodule initiation and nodule maintenance will be discussed. The extracellular domains of the trans-membrane kinases carry LysM domains suggesting that they are involved in perception of the rhizobial lipochitin-oligosaccharide signals and in deciphering the structure of lipochitin-oligosaccharides. Experiments and studies addressing these questions will be presented and the involvement of receptor kinases in the early physiological and cellular responses as well as later during nodule development will be illustrated.

PL8-3

Virus and plant endogenous siRNAs in antiviral responses and pathogen discovery

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RNA-based antiviral immunity (RVI) mediates specific virus clearance in diverse eukaryotic organisms by the cellular RNAi pathway using virus-derived siRNAs produced in response to infection. We show that effective RVI in *Arabidopsis thaliana* requires production and antiviral activities of viral secondary siRNAs in pathways involving two members from each of the Dicer (DCL4/DCL2), RNA-dependent RNA polymerase (RDR1 & RDR6) and Argonaute families (AGO1/AGO2). However, the two members of each gene family exhibit distinct antiviral activities, indicating that gene duplication is followed by functional diversification in *A. thaliana*. In addition to amplifying viral siRNAs, we found that RDR1 also mediates production of a novel class of endogenous siRNAs targeting more than a thousand of *A. thaliana* genes, suggesting a new mechanism for RDR1-dependent antiviral activity. Finally, I shall describe development of novel approaches for the discovery of viruses and viroids based on computational analyses of the total host small RNAs.

PL8-4

Plant volatiles drive ecological interaction networks

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In response to damage by herbivorous arthropods, plants emit a blend of volatiles, which are referred to herbivore-induced plant volatiles (HIPVs). Several studies have reported that blends of HIPVs are herbivore species-specific, and such specific HIPVs attracted carnivorous natural enemies of the damaging herbivores. For plants, the emission of HIPVs that attract natural enemies of herbivores is regarded as an induced indirect defence strategy when the attracted carnivores reduce the damage caused by a current herbivore infestation. For foraging carnivores, specific response to HIPVs increases their prey finding efficacy, since HIPVs indicate the presence of their prey on plants. For herbivores, HIPVs indicate the presence of con/heterospecific herbivores and natural enemies on plants. Thus, some herbivores avoid HIPVs that indicate previously used food resources and potential enemy dense space. HIPVs can signal within an individual plant; plants increase resistance in undamaged parts when exposed to volatiles from damaged parts of themselves. Further, HIPVs emitted from infested plants induce defensive responses to neighboring intact conspecific plants. In this context, HIPVs mediate interaction between herbivore-infested plants and intact neighboring plants (plant-plant signaling). Taken together, HIPVs mediate multiple interactions and function as information in food web. We call such systems interaction/information networks. In this paper, we will report our recent studies on interaction/information networks mediated by HIPVs in tritrophic systems of (1) plants, caterpillars and parasitic wasps, and (2) plants, spider mites and predatory mites.

ABSTRACTS

Concurrent Sessions

CS01-1

Patterns and receptors in Arabidopsis immunity

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Host pattern recognition receptor-mediated perception of microbe-associated molecular patterns (MAMP) is a prerequisite for the initiation of antimicrobial defenses in all multicellular organisms including plants. As metazoans, plants have evolved immune receptors for the recognition of bacterial lipopolysaccharides, flagellin and peptidoglycan. Here, I will report on the identification of a plant peptidoglycan receptor complex mediating peptidoglycan sensing and immunity to bacterial infection, and will discuss convergent evolution of peptidoglycan recognition receptors across lineage borders.Immunity to bacterial infection is not only the result of microbial pattern recognition, but may also be brought about upon recognition of host derived damage-associated microbial patterns. Experimental evidence will be presented for how a microbial pore-forming toxin resembling aquaporins mediates microbial attack and plant immunity. Phytotoxin-induced cellular damage-associated activation of plant immunity is reminiscent of microbial toxin-induced inflammasome activation in vertebrates and, thus, constitutes another conserved element in animal and plant innate immunity.

CS01-2

OsRLCK2 targeted by *Xanthomonas* Xoo1488 effector regulates MAP kinase cascade activated by OsCERK1-mediated recognition of chitin in rice

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Plant bacterial pathogens equipped with the type III secretion system (TTSS) generally deliver different TTSS effector proteins into plant cells. These TTSS effector proteins modulate the function of crucial host regulatory molecules and allow bacteria to invade plant cells. To identify Xoo TTSS effectors that inhibit host immune responses, we generated transgenic rice plants expressing each of 10 effectors of *Xanthomonas oryzae* pv. *oryzae* (Xoo). Among them, the transgenic rice plants expressing Xoo1488 showed severe susceptibility to the TTSS-deficient mutant of Xoo. Over-expression of Xoo1488 also suppressed chitin-induced immune responses including MAP kinase activation and defense gene expression in rice, suggesting that Xoo1488 may inhibit host factors involved in chitin-triggered resistance. We identified OsRLCK1 and OsRLCK2 encoding receptor like cytoplasmic kinases as potential targets of Xoo1488. OsRLCK1 and OsRLCK2 are grouped into the RLCK VII subfamily and localized at plasma membrane. BiFC experiments indicated that OsRLCK2 interacted with chitin receptor OsCERK1 at plasma membrane. The interaction between OsRLCK2 and OsCERK1 was also confirmed by Co-IP and two-hybrid experiments. Phosphorylation of OsRLCK2 was induced at 5 min after chitin treatment, which preceded MAP kinase activation. In addition, over-expression of OsRLCK2 enhanced chitin-induced MAP kinase activation, suggesting that OsRLCK2 functions upstream of MAP kinase cascade. We have also identified rice MAPKKK interacted with OsRLCK2. Thus, it is possible that OsRLCK2 may transmit a signal from OsCERK1 to the downstream MAPKKK in chitin-induced immunity.

CS01-3

Identification of a receptor-like kinase (RLK) required for functionality of receptor-like proteins (RLPs) involved in pathogen resistance of tomato

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Cf and Ve1 are membrane-anchored receptor-like proteins (RLPs) that mediate tomato resistance to the fungal pathogens *Cladosporium fulvum* and *Verticillium dahliae*, respectively. Since the identification of the first RLP (Cf-9; Jones et al., Science (1994) 266: 789-7931), the mechanism by which these extracellular receptors activate cytoplasmic signalling has remained elusive. As RLPs lack a cytoplasmic signalling domain, we anticipate recruitment of a co-receptor with a signalling domain, such as a kinase (Joosten and De Wit, Ann. Rev. Phytopathol. (1999) 37: 335-367). To identify such a co-receptor, we immunopurified transiently expressed Cf-4-eGFP and Ve1-eGFP fusion proteins from *Nicotiana benthamiana*, followed by mass spectrometry. Indeed, we identified a receptor-like kinase (RLK) that interacts with both Cf-4 and Ve1. When the *RLK* gene is silenced in *N. benthamiana*, the Cf-4/Avr4-triggered hypersensitive response (HR) is compromised. Ve1 provides *Verticillium* resistance in Arabidopsis (Fradin et al., Plant Physiol. (2011) 156: 2255-2265), which contains a homologue of this RLK. Interestingly, Ve1 function is lost in *RLK* knock-out mutants, as these plants are fully susceptible to *Verticillium*. Future studies are aimed at elucidating the exact role of this RLK in Cf and Ve1 function. Besides this RLK, we identified endoplasmic reticulum (ER) HSP70 binding proteins (BiPs) and lectin-type calreticulins (CRTs), which are chaperones involved in ER-Quality Control (ER-QC). Interestingly, silencing of *CRT3a* resulted in loss of full resistance to *C. fulvum*. We found that the Cf-4 protein still normally accumulates, however the pool of mature Cf-4 protein carrying complex-type *N*-linked glycans is largely reduced.

CS01-4

Identification of innate immunity elicitors using molecular signatures of natural selection

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The innate immune system is an ancient and broad-spectrum defense system found in all eukaryotes. The detection of microbial elicitors results in the up-regulation of defense-related genes and the elicitation of inflammatory and apoptotic responses. These innate immune responses are the front-line barrier against disease because they collectively suppress the growth of the vast majority of invading microbes. Despite their critical role, we know remarkably little about the diversity of immune elicitors. To address this paucity, we reasoned that hosts are more likely to evolve recognition to “core” pathogen proteins under strong negative selection for the maintenance of essential cellular functions, whereas repeated exposure to host-defense responses will impose strong positive selective pressure for elicitor diversification to avoid host recognition. Therefore, we hypothesized that novel bacterial elicitors can be identified through these opposing forces of natural selection. We tested this hypothesis by examining the genomes of 46 bacterial phytopathogens and identifying strong candidate elicitors

that have an excess of positively selected residues in a background of strong negative selection. We show that these positively selected residues are atypically clustered, similar to patterns seen in the few well-characterized elicitors. We then validated selected candidate elicitors by showing that they induce *Arabidopsis thaliana* innate immunity in functional (virulence suppression) and cellular (callose deposition) assays. These finding provide targets for the study of host-pathogen interactions and applied research into alternative antimicrobial treatments.

CS01-5

Bacterial effector manipulates JAZ transcription repressors of jasmonate signaling to facilitate bacterial infection

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Many Gram negative phytopathogenic bacteria inject an array of type III secreted effectors (T3SEs) into plant cells via the type III secretion system (T3SS). After entering the host cytosol, T3SEs associate with specific host targets and facilitate pathogen infection. Many of these host targets are key components of plant immunity. Here, we will report our recent findings that the *Pseudomonas syringae* T3SE HopZ1, directly targets jasmonate-ZIM-domain (JAZ) proteins in the natural host soybean and the model plant *Arabidopsis thaliana*. JAZs are key negative transcription regulators of jasmonate (JA)-responsive genes and major components of the jasmonate receptor complex. During infection, *P. syringae* producing HopZ1 induces the degradation of JAZ proteins and activates the expression of JA-responsive genes. Importantly, HopZ1 could partially rescue the virulence defect of *P. syringae pv. tomato* (*Pto*) strain DC3118, a mutant that does not produce the JA-mimicking phytotoxin coronatine. This is a novel example by which a bacterial effector directly manipulates the core regulators of phytohormone signaling to facilitate infection. Targeting of JAZ repressors by both coronatine toxin and HopZ1 effector suggests that the JA receptor complex is potentially a major hub of host targets for bacterial pathogens. Recent progress on the mechanisms underlying HopZ1-mediated JAZ degradation will be discussed.

CS01-6

Lectin receptor kinases as modulators of the Arabidopsis innate immunity response

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Diseases caused by microbial pathogens significantly contribute to the overall loss in crop yield worldwide. In order to better understand plant resistance to deleterious pathogens, my laboratory uses the priming agent beta-aminobutyric acid (BABA) as a tool to discover new genes involved in the Arabidopsis defense response. Lectin receptor kinases play important role in animal innate immunity, but their possible involvements in plant resistance to pathogen remain largely elusive. Using a reverse genetic approach in *Arabidopsis thaliana*, we demonstrated that the BABA-responsive L-type lectin receptor kinase-VI.2 (LecRK-VI.2) contributes to disease resistance against the hemi-biotrophic *Pseudomonas syringae* and the necrotrophic *Pectobacterium carotovorum* bacteria. Notably, LecRK-VI.2 is required for full activation of the pattern-triggered immunity (PTI) response. Overexpression studies combined with genome-wide microarray analyses indicated that LecRK-VI.2 positively regulates the PTI response. LecRK-VI.2 is also required for full BABA-induced resistance and priming of PTI. Our data indicate that LecRK-VI.2 is a novel mediator of the Arabidopsis PTI response and provides insight into molecular mechanisms governing priming. We will provide novel information on the role of lectin receptor kinases in Arabidopsis innate immunity, with emphasizes on stomatal immunity.

CS02-1

***inhospitable*, a novel rice mutant abolishes hyphopodia formation by arbuscular mycorrhizal fungi**

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The arbuscular mycorrhiza (AM) is an ancient symbiosis between most land plants and glomeromycotan fungi that is based on the mutual exchange of nutrients between the two partners. Arbuscular mycorrhizal colonization is initiated by an exchange of signals involving plant strigolactones and fungal signals called myc factors. Following recognition AM fungi form a hyphopodium at the root surface, enter the root cortex and form branched arbuscules inside cortex cells. Plant proteins required for intraradical colonization and arbuscule formation have been identified. In contrast, factors that govern hyphopodium formation, which marks the earliest step of physical contact between the symbionts, remain largely unknown. Investigating the role of JA for AM colonization of rice we found, that the JA-deficient mutant *hebiba* does not support hyphopodia formation. The *hebiba* mutation was mapped to a genomic deletion of 170 kb containing a JA biosynthesis gene. Complementation of the mutant with exogenous JA or the deleted JA biosynthesis gene did not restore AM colonization. The AM phenotype of *hebiba* is therefore due to the deletion of a novel gene, which we called *Inhospitable* (*IHO*). Progress on the identification of the *IHO* gene and further characterization of the mutant phenotype will be presented.

CS02-2

HAR1, KLAVIER and TOO MUCH LOVE mediate CLE peptide signaling in long-distance control of nodulation

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To keep the symbiotic balance with rhizobia, legumes evolved specific mechanisms to control the nodule number in response to internal and external cues. An important internal cue is a feedback regulatory system involving long-distance signaling also known as autoregulation of nodulation (AON). AON is believed to consist of two presumptive long-distance signals, i.e., the root-derived and shoot-derived signals. The root-derived signal is thought to be generated in roots in response to rhizobial Nod factors and then translocated to the shoot, while the shoot-derived signal is generated in shoots and then translocated to the root to restrict further nodulation. Mutants defective in AON display a 'hyper-nodulation' phenotype. Using *Lotus japonicus* ecotypes, Gifu B-129 and Miyakojima MG-20, we have isolated *har1*, *klavier*, *too much love* (*tml*) and *plenty* hyper-nodulation mutants. Grafting experiments revealed that HAR1 and KLAVIER function in the shoot while TML and PLENTY in the root. *HAR1* encodes a LRR receptor-like kinase that shows the highest similarity with Arabidopsis *CLV1* that maintains shoot apical meristem by receiving a CLE peptide derived from the stem cell region. *KLAVIER* is also indispensable for AON signaling and encodes a LRR receptor-like kinase. The mutation exhibits stem fasciation as well as hyper-nodulation phenotype. On the other hand, TML is likely to function downstream of HAR1, possibly as a receptor or a mediator of the as-yet unidentified shoot-derived signal. Two CLE peptides (LjCLE-RS1, -RS2) are strong candidates of the root-derived signal. Overexpression of *CLE-RS1/2* inhibits nodulation systemically in HAR1, KLAVIER and TML-dependent manner.

CS02-3

Activation of the host symbiosis signaling by rhizobial type III secretion system

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Root nodule symbiosis between leguminous plants and nitrogen-fixing bacteria (rhizobia) requires molecular communication between both partners. Key components for the establishment of symbiosis are host plant-derived flavonoids that induce the transcription of rhizobial nodulation (*nod*) genes and rhizobium-produced lipochitooligo-saccharides (Nod-factors) that initiate nodule development and bacterial entry. Besides the Nod-factors there are other determinants that influence the extent of the symbiosis. Among them, we have focused on a rhizobial protein secretion system, called type III secretion system (T3SS). In this study, we analyzed the role of T3SS in the interaction between *Bradyrhizobium elkanii* and soybean (*Glycine max* (L.) Merr.).Mutational analysis and inoculation tests of B. elkanii USDA61 revealed that the presence of T3SS affected symbiotic capacity either positively or negatively depending on host genotype. On G. max cv. Enrei, wild-type USDA61 induced more nodules than T3SS mutant. On the other hands, cultivar Hill interdicted nodulation by the wild type but was nodulated by the T3SS mutant. Intriguingly, when infected to the soybean mutant En1282 that has defective Nod factor receptor 1 (NFR1) and show non-nodulating phenotype with *B. japonicum* and other rhizobial strains, USDA61 but not its T3SS mutant induced effective nodules. Transcriptional analysis revealed that the expression of early nodulation gene *ENOD40* and *NIN* was increased in the root of En1282 inoculated with wild type but not with T3SS mutant. These results suggest that T3SS of USDA61 has functions to enforce legume host to initiate symbiotic programs by bypassing Nod-factor recognition.

CS02-4

Studies on putative type III-secreted effector proteins containing a self-cleavable DUF1521 domain

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Bradyrhizobium japonicum is a symbiont of soybean and secretes proteins whose synthesis is induced by the isoflavone genistein. Two of these type III-secreted proteins are the homologs NopE1 and NopE2, which exhibit 77% sequence identity. In plant experiments, it was shown that the proteins affect nodulation positively or negatively depending on the host (1). Reporter assays revealed that NopE1 and NopE2 are translocated into the plant cell. Both proteins contain two similar domains of unknown function (DUF1521). NopE1 and truncated derivatives were expressed in *E. coli* as GST fusion proteins and purified with glutathione sepharose affinity chromatography. NopE1 contains an autoproteolytic cleavage site between an aspartate and proline within each of the DUF1521 domains (1). Self-processing of the protein can be induced by calcium and is prevented by calcium chelators (2). Experiments with truncated derivatives show that the minimal domain required for autocleavage is the DUF1521 domain. Under native conditions, NopE1 forms dimers and the fragmented protein parts adhere to each other. Database searches indicate the presence of the DUF1521 domain in proteins from different Proteobacteria, e.g. *Vibrio coralliilyticus* and *Burkholderia phytofirmans*. The DUF1521 domain-containing protein of *V. coralliilyticus* exhibits a similar self-processing activity as NopE1. Therefore, this domain probably serves a function in several non-

related interactions between bacteria and their eukaryotic host. (1) Wenzel et al. (2010). Mol. Plant-Microbe Interact. 23:124-129; (2) Schirrmeister et al. (2011). J. Bacteriol. 193:3733-3739.

CS02-5

Zwitterionic membrane lipids phosphatidylethanolamine and phosphatidylcholine affect transcription and physiology of *Sinorhizobium meliloti* in different ways

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Sinorhizobium meliloti contains the negatively charged phosphatidylglycerol and cardiolipin as well as the zwitterionic phosphatidylethanolamine (PE) and phosphatidylcholine (PC) as major membrane phospholipids. In previous studies we had isolated *S. meliloti* mutants that lack PE or PC. Transcript profiles of mutants unable to form PE or PC are distinct; they differ from each other and they are different from the wild type. For example, a PE-deficient mutant of *S. meliloti* shows an increase of transcripts that might be required for the degradation of C1 compounds and a decrease of transcripts that might be required for iron uptake or for the catabolism of myo-inositol. In contrast, a PC-deficient mutant of *S. meliloti* shows an increase of transcripts that encode a possible lytic transglycosylase or enzymes required for succinoglucan biosynthesis and a decrease of transcripts that are required for flagellum formation. Changes similar to those in the PC-deficient mutant are observed when *S. meliloti* wild type is exposed to acidic conditions of growth. Growth of the PC-deficient mutant is especially sensitive to acidity and we suggest that a PC-deficient membrane in *S. meliloti* is more fluid and therefore more permeable for protons. Also, some mutants altered in the ExoR/ExoS/ChvI regulatory system resemble the PC-deficient mutant in their transcript profile and we suggest that the lack of PC in the sinorhizobial membrane is sensed and transmitted by the ExoR/ExoS/ChvI regulatory system.

CS02-6

Sinorhizobium meliloti* ECF sigma factors are required for symbiosis on *Medicago sativa* and *M. truncatula

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Transcriptional regulation is a key feature of *Sinorhizobium meliloti* adaptation to the plant environment (1). RNA polymerase sigma subunits provide a mechanism to control transcription at a global scale by determining promoter specificity. The *S. meliloti* genome encodes 9 ECF-like sigma factors that fall into major ECF families (2) ECF26, 15, 16, 29, and 41. The *fecI* gene is ECF-like but uncategorized. Another locus, Smc01150, probably encodes an ECF42-like sigma. Using the neomycin-resistance insertional vector pVO155 (3) and a hygromycin-resistant variant, we constructed single and double mutants for the ECF sigma factor genes *rpoE1-rpoE9* and *fecI*. All single and double mutants were prototrophic and showed normal motility and EPS production; however, they all showed some degree of enhanced sensitivity to the detergent DOC. The 10 single mutants all appeared normal for symbiosis, as judged by nodule formation, nodule appearance, and rate of acetylene reduction. Among the 45 double mutants, 32 established normal symbiosis with both *Medicago sativa* and *M. truncatula*. However, 13 were abnormal: 4 were Nod $^{-}$, and another 9 were Nod $^{+}$Fix $^{-}$. A few of the ECF double-mutants were normal on one host plant, but defective on the other. We assayed *nod* gene expression and visualized bacterial invasion to define potential developmental events that require action of these alternative sigma factors. (1) Barnett and Fisher (2006) Symbiosis 42:1-24; (2) Staron et al (2009) Molec. Microbiol. 74:557-581; (3) Oke and Long (1999) Molec. Microbiol 32:837-849.

CS03-1

A novel component of the Prp19-associated complex is essential to safeguarding efficient intron splicing of pathogenicity genes in the rice blast fungus

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The spliceosomes of higher eukaryotes usually contain additional components that are absent in *Saccharomyces cerevisiae*. However, few of them have been functionally characterized. We isolated a novel gene named *PCG1* that is essential to pathogenicity of the model phytopathogenic fungus *Magnaporthe oryzae* and encodes such a splicing factor. Deletion of PCG1 resulted in loss of pathogenicity and intron retention in transcripts for thousands of genes. Interestingly, 55 genes required or important for pathogenicity were found to have intron retention in their transcripts in the *PCG1* deletion mutant. Pcg1 was co-immunoprecipitated with dozens of components of the spliceosome, and physically interacted with several components of the Prp19-associated complex, notably with Cwc2 that is required for intron splicing. Deletion of *FgPCG1*, the ortholog in the wheat scab fungus, also resulted in loss of pathogenicity. Introduction of *FgPCG1* and the human ortholog *hCCDC12* could completely and partially rescue the defects caused by *PCG1* deletion, respectively. Thus, Pcg1 and its orthologs in higher eukaryotes are an important component of the Prp19-associated complex and are essential to safeguarding efficient intron splicing of pathogenicity genes in fungal pathogens. This study also provided new insights into protein interaction networks of the Prp19-associated complex.

CS03-2

ChTn1, a Tc1-mariner transposable element of *Cochliobolus heterostrophus* is regulated by intron retention

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The *Cochliobolus heterostrophus* genome carries class II transposable elements of the *Tc1-mariner* superfamily. One of these transposable elements named *ChTn1* has been analyzed and found to occur as nine complete copies. The sequences fall into five subfamilies based on nucleotide identity. Comparison of different sequences revealed the presence of a RIP-like process. The majority of the *ChTn1* sequences are flanked by genes and three of them are very close to promoter sequences. Analysis of different strains of *C. heterostrophus* showed polymorphism in hybridization profiles, but no footprints were found at sites that showed absence of the element, indicating that there was no integration of elements in these positions or the excisions were perfect. *ChTn1-like* sequences are present in *Alternaria brassicicola*, *Pyrenophora tritici-repentis*, *Pyrenophora teres*, *Cochliobolus carbonum*, *Cochliobolus sativus*, and *Setosphaeria turcica* and thus conserved in Dotheomycetes. The Analyses of the *ChTn1* sequences indicate introns can be retained and that two transcripts are produced and translated into two polypeptides with 128 aa and 436 aa (transposase). These two polypeptides may compete for binding sites in the TIR sequences. This is the first report where intron retention is shown as a possible regulatory mechanism in transposable elements of fungi.

CS03-3

Roles of histone lysine methyltransferases in the pathogenicity of *Magnaporthe oryzae*

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The rice blast fungus *Magnaporthe oryzae* shows dramatic morphological changes during infection with global transcriptional alterations possibly resulted from genome-wide chromatin remodeling. Here we report genetic dissection of histone methyltransferase (HMT) genes in *M. oryzae*. BLAST searches against the *M. oryzae* genome identified seven putative histone lysine methyltransferase genes, which we named MoHMT1 to 7. Using a wheat-infecting strain of *M. oryzae*, we constructed knock-out (KO) mutants of the seven MoHMT genes by the split-marker recombination method. Western blotting analysis of histone protein in the KO mutants revealed that MoHMT1 was associated with methylation of histone H3 lysine 9 (H3K9me), and MoHMT4 was responsible for H3K4me. Some of the MoHMT-KO mutants showed defects in vegetative growth, conidiation, appressorium formation, and pathogenicity at variable levels. Remarkably, MoHMT4-KO mutants were severely impaired in appressorium formation and completely lost pathogenicity on the original host wheat, indicating that H3K4me is an important epigenetic mark for infection-related gene expression in *M. oryzae*. Appressorium formation was greatly restored in the MoHMT4-KO mutants by exogenous addition of cAMP or the cutin monomer 16-hydroxypalmitic acid, suggesting that MoHMT4 might be involved in signal perception leading to appressorium formation. Interestingly, the MoHMT4-KO mutants were still infectious on the susceptible barley cultivar Nigrate, suggesting its role in overcoming some host-specific resistance. Chromatin immunoprecipitation (ChIP) and ChIP-seq analyses revealed dynamic changes in distribution patterns of H3K4me in the *M. oryzae* genome during infection.

CS03-4

A refinement of the predicted secretome for the wheat leaf pathogen *Mycosphaerella graminicola*

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The infection of wheat leaves by the fungus *Mycosphaerella graminicola* involves an initial extended period of symptomless intercellular colonisation prior to the development of disease lesions. Previous functional genomics and gene expression profiling studies have implicated the production of secreted virulence effector proteins as a key component facilitating the initial symptomless growth phase (1, 2). With a view to identifying further candidate virulence effectors, we have re-analysed the predicted protein secretome from this pathogen, by combining several bioinformatic approaches aimed to increase the probability of identifying truly secreted proteins. An initial secretome of 970 proteins was predicted and a further prediction of 556 was made based upon further stringent selection criteria deriving from WolfPsort protein localisation prediction. Of these, 298 possess some functional annotation (based upon PFam; KOG or the CDD databases) leaving 258 with no functional annotation. Further characterisation of the un-annotated proteins included the analysis of features associated with known fungal effectors, for example, small size, cysteine-rich, and Blastp searches performed against other sequenced fungal genomes. Finally evidence in support of gene prediction was derived from gene expression profiling during fungal growth in vitro and in planta. Subsets of candidate genes are currently being subjected to sequence analysis, reverse genetics and BSMV-mediated overexpression in wheat leaves. (1) Marshall et al., (2011). Plant Physiol. 156, 756-769; (2) Rudd et al., (2010) Fungal Genet Biol. 47, 19-32.

CS03-5

Septin-mediated plant cell invasion by the rice blast fungus *Magnaporthe oryzae*

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To cause rice blast disease, the fungus *Magnaporthe oryzae* develops a pressurized dome-shaped infection structure called an appressorium, which physically ruptures the rice leaf cuticle to gain entry to plant tissue. The metabolic changes and MAPK signalling pathways accompanying appressorium development have been widely studied over the last decade. However, we have very little information regarding the cell biology of infection, and in particular the breaking of cellular symmetry during appressorium repolarization. One of the striking features of appressorium-mediated infection is the generation of an 8 MPa turgor pressure and its conversion into mechanical force sufficient to break the plant cuticle. Given that there is no melanin layer at the site of penetration, it is intriguing to know how the plasma membrane maintains integrity at this level of pressure. In this report, we will present a hitherto unknown scaffold around appressorium pore, which is mainly composed of septins and actin. Furthermore, we will provide data about the organization of septin/actin rings, their relationship with plasma membrane and their roles in maintaining cortical rigidity and polarity establishment. Also, we will discuss how septins control diffusion of proteins involved in penetration peg emergence. Additionally we will show turgor pressure acts as a signal for formation of septin/actin rings. Finally, we will propose a model, which, we believe, will significantly increase our understanding of appressorium function.

CS03-6

Pathogenesis and infection related morphogenesis of *Colletotrichum orbiculare*

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Colletotrichum species form well-developed infection structure named appressoria as a host invasion structure. They are generally melanized single cells developed from germ tubes from conidia. By forward genetics approach, factors involved in appressorium development of *Colletotrichum orbiculare* were extensively analyzed. Findings on morphological and functional development of infection structure in *Colletotrichum orbiculare* will be presented. **Signal transduction** : MAPK and cAMP signaling pathways are linked to infection-related morphogenesis. Recently, we identified a gene *CoIRA1* coding for a predicted protein with RAS GTPase-activating domain, which presumably controls RAS, upstream of cAMP signaling based on the *S. cerevisiae* homologs function. The coira1 mutant showed attenuated infection related morphogenesis. **Peroxisome function** : Peroxisome biogenesis genes of which roles in pathogenesis have been elucidated include *CoPEX6* and *CoPEX13* . We identified a novel peroxin gene *CoPEX22* that shuttles between peroxisome and Woronin body, a peroxisome derived cellular organelle that function for sealing of septal pore when the fungal spore damaged. The *copex22* mutant showed attenuated appressorium development. **Cellular polarity** : Kelch motif containing genes *CoKEL1* and *CoKEL2* are involved in appressorium development. Cellular location of Cokel1 and Cokel2 proteins is microtubule dependent fashion. The gene disrupted mutants form aberrant appressoria which accompanies defectiveness in further development of infection hyphae. Recently, we identified *CoBUB2* , a *S. cerevisiae BUB2* homolog that constitutes a checkpoint of mitotic exit named SPOC (spindle position checkpoint). cobub2 mutants showed attenuated infection related morphogenesis and deficiency in proper nuclear division. Organized control of mitosis and cell polarity would be essential for proper appressorium morphogenesis.

CS04-1

Nonhost interactions between *Arabidopsis* and anthracnose fungi

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Arabidopsis thaliana exhibits durable resistance, called nonhost resistance, against non-adapted *Colletotrichum* species that cause anthracnose disease on other plants. We have reported that both PEN2-dependent antifungal metabolite and EDR1-dependent antifungal peptide pathways are involved in preventing entry of a non-adapted *Colletotrichum gloesporioides* (*Cg*). Here, we show that GSH1/PAD2 is required for both pre-invasive and post-invasive defense in nonhost interaction of *Arabidopsis* with *Colletotrichum* species. *GSH1* encodes γ-glutamylcysteine synthetase critical for biosynthesis of glutathione (GSH). Inoculation assay of *Cg* on *gsh1* mutants with or without GSH showed that biosynthesis of GSH is required for pre-invasive resistance against *Cg*. The genetic analysis of *pen2 gsh1* suggests that a defect in pre-invasive defense in *gsh1* is mainly due to reduction in biosynthesis of PEN2-related antifungal metabolites in response to *Cg*. In contrast to *pen2*, *gsh1* mutants permitted subsequent post-invasive growth of *Cg*, suggesting that GSH1 is critical for post-invasive resistance that accompanies cell death response. Phenotypic analysis of series of mutants such as *pen2 pad3* and *cyp79B2 cyp79B3* mutants suggests that tryptophan-derived antifungal metabolites, including a camalexin, are involved in post-invasive resistance against *Cg*. The adapted *C. higginsianum* infects the ecotype Col-0 of *Arabidopsis* but cannot infect the ecotype Ws-0 because Ws-0 has a functional dual *R* gene set (*RPS4* and *RRS1*). Notably, *gsh1* or *cyp79B2 cyp79B3* impaired the dual *R* gene resistance against *C. higginsianum*. These results indicate that GSH-mediated synthesis of tryptophan-derived antifungal metabolites is required for hypersensitive response of *Arabidopsis* against both adapted and non-adapted anthracnose fungi.

CS04-2

RAC/ROP G-protein interacting proteins of barley are involved in microtubule organization and basal resistance to penetration by the barley powdery mildew fungus

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Little is known about the nature and function of host susceptibility factors. The barley RAC/ROP G-protein RACB is required for full susceptibility to the powdery mildew fungus, *Blumeria graminis* f.sp. *hordei*, and it is involved in cell polarity and cytoskeleton organization. We identified a novel microtubule associated RAC/ROP-GTPASE ACTIVATING PROTEIN (MAGAP1) and a ROP binding cytoplasmic kinase (RBK1) interacting with RACB in yeast and in planta. Fluorescent MAGAP1 decorated cortical microtubules and can be recruited by constitutively activated CA RACB to the plasma membrane. CA RACB supported fungal entry and might destabilize microtubules, possibly releasing MAGAP1 for negative feedback regulation. Under fungal attack, cortical microtubules strongly polarized to sites of successful defence at cell wall papillae. In contrast, microtubules locally loosened when the fungus succeeded in penetration. Overexpression of MAGAP1 supported focal polarization of microtubules to sites of fungal attack. RNAi targeting MAGAP1 or RBK1 supported susceptibility to penetration by *B. graminis*, whereas over-expression of MAGAP1 limited fungal entry. Accordingly, a dominant negative variant of MAGAP1 supported fungal penetration success. RNAi of RBK1, which can be activated by active RAC/ROPs in vitro, destabilized cortical microtubules. Data suggest that function of RACB might involve reorganization of microtubules, which is under antagonistic or additional control of MAGAP1 and RBK1. Results add to our understanding of how intact plant cells accommodate biotrophic infection structures and establish RACB, MAGAP1 and RBK1 as players in re-organization of microtubules under fungal attack.

CS04-3

Mechanisms of secretion and delivery of rice blast effector proteins into live rice cells

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Blast disease, caused by *Magnaporthe oryzae*, remains a threat to global rice production, and has recently emerged as a threat to global wheat production. During biotrophic invasion, *M. oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the rice cytoplasm, and apoplastic effectors, which are retained in the extracellular compartment between the fungus and the rice plasma membrane. BICs localize adjacent to tips of filamentous hyphae that enter rice cells, and remain beside the first-differentiated bulbous invasive hyphal cells. In contrast, secreted apoplastic effectors uniformly outline bulbous invasive hyphae that grow to fill the invaded cell. Chimeric gene analyses indicate that effector promoters play a major role in determining preferential localization of cytoplasmic effectors in BICs. Consistent with this, a cytoplasmic effector is strongly up-regulated in the BIC-associated hyphal cells. Live cell microscopy of invasive hyphae expressing fluorescent secretion machinery components confirmed distinct growth and secretion patterns for the filamentous and bulbous invasive hyphae, and suggested that secretion into BICs continued while invasive hyphae grew elsewhere in the host cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors into BICs. Pathogen mutants that failed to express exocyst complex components or a t-SNARE were defective in secretion of BIC-localized effectors. Our data suggest that exocyst and SNARE complexes play a role in secretion of cytoplasmic effectors into BICs by an unconventional, Golgi-independent secretory pathway.

CS04-4

***Phytophthora* effectors facilitate infection by suppressing host RNA silencing**

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Effectors are essential virulence proteins produced by a broad range of parasites including viruses, bacteria, fungi, oomycetes, protozoa, insects and nematodes. Upon entry into host cells, effectors manipulate specific physiological processes or signaling pathways to subvert plant immunity. So far, the majority of effectors, especially those produced by eukaryotic pathogens, remain functionally uncharacterized. Here we show that two *Phytophthora* RxLR effectors suppress RNA silencing in plants by inhibiting the biogenesis of small RNAs. Ectopic expression of either of the two *Phytophthora* suppressors of RNA silencing (PSRs) significantly enhances the susceptibility of *Nicotiana benthamiana* to infection with potato virus X or *Phytophthora infestans*. Although PSR2 specifically suppresses the biogenesis of small interference RNA, PSR1 represents the first example of non-viral pathogen effectors that is able to suppress both microRNA and small interference RNA pathways. These data demonstrated that *Phytophthora* have evolved effectors to manipulate host RNA silencing processes

through distinctive mechanisms in order to facilitate infection. The identification and characterization of the PSRs, and recent findings on the molecular mechanisms by which PSRs suppress plant RNA silencing will be presented.

CS04-5

Isolation and functional characterization of the host targets of *Phytophthora infestans* RXLR effector Avr-chc1

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Recently, a large new family of late blight *R* genes *(Rpi)* was characterised from south American species like *Solanum chacoense* and *Solanum berthaultii* and is referred to as the *Rpi-chc1* gene family. The corresponding *Avr* genes, referred to as *Avr-chc1*, were identified and also they belong to a large effector gene-family from *Phytophthora infestans*. Different members of the *Rpi-chc1* family can recognise overlapping- but also non overlapping sets of *Avr-chc1* family members. This illustrates the co-evolution between pathogen and host and suggests that the Rpi-chc1 proteins guard one or more important virulence target(s) or susceptibility factor(s). In order to identify this target(s), we use pull-down assays following *in planta* expression of epitope-tagged Avr-chc1 proteins to isolate co-purifying host proteins. Using LC MS co-purifying peptides were identified and they corresponded to putative effector targets which were either specific or common for one or more Avr-chc1 members. As putative targets we identified one protein of the CC-NB-LRR type in addition to several proteins essential for vesicle trafficking. Currently, co-immunoprecipitation and yeast two-hybrid assays are used to confirm the interaction between the identified target(s) on one side and the corresponding Avr, or the corresponding R protein on the other side. The function of the identified effector target(s) in plant defence or susceptibility is studied using virus induced gene silencing (VIGS) in *Nicotiana benthamiana*. Ultimately, this knowledge will be translated into novel late blight resistance breeding strategies for potato.

CS04-6

Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species

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Magnaporthe oryzae is the causal agent of rice blast disease, a devastating problem worldwide. This fungus has caused breakdown of resistance conferred by newly developed commercial cultivars. To address how the rice blast fungus adapts itself to new resistance genes so quickly, we examined chromosomal locations of *AVR-Pita*, a subtelomeric gene family corresponding to the *Pita* resistance gene, in various isolates of *M. oryzae* and its related species. We found that *AVR-Pita* is highly variable in its genome location, occurring in chromosomes 1, 3, 4, 5, 6, 7, and supernumerary chromosomes, particularly in rice-infecting isolates. When expressed in *M. oryzae*, most of the *AVR-Pita* homologs could elicit *Pita*-mediated resistance, even those from non-rice isolates. *AVR-Pita* was flanked by a retrotransposon, which presumably contributed to its multiple translocation across the genome. On the other hand, family member *AVR-Pita3*, which lacks avirulence activity, was stably located on chromosome 7 in a vast majority of isolates. These results suggest that the diversification in genome location of *AVR-Pita* in the rice isolates is a consequence of recognition by *Pita* in rice. We propose a model that the multiple translocation of *AVR-Pita* may be associated with its frequent loss and recovery mediated by its transfer among individuals in asexual populations. This model implies that the high mobility of *AVR-Pita* is a key mechanism accounting for the rapid adaptation toward *Pita*. Dynamic adaptation of some fungal plant pathogens may be achieved by deletion and recovery of avirulence genes using a population as a unit of adaptation.

CS05-1

Regulation of bioprotective metabolite biosynthesis in the grass symbiont *Epichloe festucae*

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Epichloe festucae in association with perennial ryegrass synthesizes a range of secondary metabolites that confer bioprotective benefits to the grass host. These include peramine a potent insect feeding deterrent and indole-diterpenes, a structurally diverse group of metabolites best known as inhibitors of mammalian large conductance Ca2+-gated K+ (BK) channels. A single non-ribosomal peptide synthetase enzyme, encoded by *perA*, is proposed to catalyse all the steps required for the synthesis of peramine. By contrast a cluster of up to 11 genes is required for the synthesis of lolitrem B, the main indole-diterpene product synthesized by *E. festucae* strain Fl1. The *ltm* locus in Fl1 is subtelomeric with the 11 genes organised into three sub-clusters separated by large blocks of type I and type II transposon relics. Both *perA* and *ltm* genes are preferentially expressed *in planta*, suggesting that plant signaling is important for de-repression of these biosynthetic pathways. The aim of this work is to determine the mechanism underlying the preferential activation of these genes *in planta*. To achieve this goal we are using a number of different approaches including: (i) a comparative analysis of promoter regions of different strains to identify putative regulatory elements, (ii) deletion analysis of promoter-*gusA* reporter constructs, (iii) metabolite activation of a promoter-*gfp* knock-in ex planta, (iv) targeted deletion of genes involved in chromatin remodeling, and (v) RNAseq analysis of wild-type versus mutant endophyte-grass symbiota. Insights gained into regulation of these bioprotective symbiotic genes from this combined approach will be presented.

CS05-2

Effect of colonization of endophytic bacteria on rice

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Various kinds of fungal and bacterial endophytes are isolated form various plants. Some endophytes have the preferable effects on the host plants, such as growth promotion, disease resistance, and drought resistance. An endophytic bacterium *Azospirillum* sp. strain B510 from surface-sterilized stems of field-grown rice was expected to have some benefits to the host rice plants. Inoculation experiments with *Azospirillum* sp. strain B510 were conducted in pots in a greenhouse, and in paddy fields in Hokkaido, Japan. B510 significantly enhanced the growth of newly generated leaves and shoot biomass under greenhouse conditions. When rice seedlings were treated with $1x10^8$ CFU ml-1, then transplanted to paddy fields, tiller numbers and seed yield significantly increased. We also analyzed the effects of *Azospirillum* sp. B510 on disease resistance in host rice plants, resulting the induction of disease resistance in rice against rice blast disease and rice bacterial blight disease. Analyzing the levels of stress-related phytohormones and expression of defense-related genes indicated the possibility that strain B510 is able to induce disease resistance in rice by activating a novel type of resistance mechanism independent of SA-mediated defense signaling. The detailed mechanisms of plant growth promotion and resistance induction by strain B510 are under investigation.

CS05-3

ppGpp controlled by the Gac/Rsm regulatory pathway sustains biocontrol activity in *Pseudomonas fluorescens* CHA0

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In *Pseudomonas fluorescens* CHA0 and other fluorescent pseudomonads, the Gac/Rsm signal transduction pathway is instrumental for secondary metabolism and biocontrol of root pathogens via the expression of regulatory small RNAs (sRNAs). Furthermore, in strain CHA0, an imbalance in the Krebs cycle can affect the strain's ability to produce extracellular secondary metabolites including biocontrol factors (1). Here we report the metabolome of wild-type CHA0, a *gacA*-negative mutant, which has lost Gac/Rsm activities, and a *retS*-negative mutant, which shows strongly enhanced Gac/Rsm-dependent activities. Capillary electrophoresis-based metabolomic profiling revealed that the *gacA* and *retS* mutations had opposite effects on the intracellular levels of a number of central metabolites, suggesting that the Gac/Rsm pathway regulates not only secondary metabolism, but also primary metabolism in strain CHA0. Among the regulated metabolites identified, the alarmone ppGpp (guanosine tetraphosphate) was characterized in detail by the construction of *relA* (for ppGpp synthase) and *spoT* (for ppGpp synthase/hydrolase) deletion mutants. In a *relA spoT* double mutant, ppGpp synthesis was completely abolished, the expression of Rsm sRNAs was attenuated, and physiological functions such as antibiotic production, root colonization and plant protection were markedly diminished. Thus, ppGpp appears to be essential for sustaining epiphytic fitness and biocontrol activity of strain CHA0.
(1) Takeuchi K, Kiefer P, Reimmann C, Keel C, Dubuis C, Rolli J, Vorholt JA, Haas D. 2009. J Biol Chem 284: 34976-85.

CS05-4

Role of the root-specific transcription factor MYB72 in rhizobacteria-induced systemic resistance

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Root colonization by plant growth-promoting rhizobacteria can trigger an induced systemic resistance (ISR) that is effective against a broad spectrum of pathogens and even insects (Zamioudis et al., 2012; MPMI 25:139-150). The root-specific transcription factor MYB72 was identified as an essential component for the establishment of ISR in Arabidopsis (Van der Ent et al., 2009; Plant Physiol. 146:1293-1304). Confocal laser scanning microscopy revealed that *MYB72* is strongly activated in root epidermal and cortical cells upon colonization of the roots by ISR-inducing *Pseudomonas fluorescens* WCS417r. A survey of the Arabidopsis transcriptome linked *MYB72* expression to iron limited conditions. Here, we report that ISR-inducing rhizobacteria upregulate the iron deficiency response in roots even under non-iron-limiting conditions. We further demonstrate that WCS417r-induced *MYB72* transcription is depended on the transcription factors FIT1 (bHLH29) and bHLH38/39, which are central regulators of iron acquisition in the roots, indicating that the transcriptional regulation of MYB72 is similar to that of the iron uptake genes *FRO2* and *IRT1*. Microarray analysis of the MYB72-dependent root transcriptome revealed a small number of upregulated genes that may be involved in the generation or translocation of a systemic ISR signal. In addition, a large cluster of MYB72-dependent genes were downregulated by WCS417r, the majority of which are defense-related. We conclude that WCS417r hijacks the iron-deficiency response of Arabidopsis, resulting in a MYB72-dependent attenuation of local immune responses to establish successful root infections. Accordingly, active root colonization by WCS417r was found to be impaired in the *myb72* mutant.

CS05-5

***Paenibacillus polymyxa* M-1, a plant growth promoting rhizobacterium, is capable of colonizing the roots of wheat**

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Strain M-1 isolated from surface sterilized wheat root tissues was identified by 16S rRNA gene sequencing and by physiological and biochemical analysis as being *Paenibacillus polymyxa*. Not only can *Paenibacillus polymyxa* M-1 promote the growth of *Lemna minor* ST, *Arabidopsis*, *Zea mays* and *Triticum aestivum*, but also can suppress wheat sharp eyespot disease which is a serious soil-borne disease on wheat caused by *Rhizoctonia cerealis*. Besides *Rhizoctonia cerealis*, *P. polymyxa* M-1 inhibited the growth of several phytopathogenic fungi (*Alternaria sonali*, *Rhizoctonia cerealis*, *Fusarium oxysporum*, *Fusarium solani*, *Botrytis cinerea*, *Gaeumannomyces graminis*, *Magnaporthe grisea* and *Phytophthora infestans*) and bacteria (*Erwinia amylovora* and *Erwinia carotovora*) *in vitro* by producing antibiotics, including fusaricidin and polymyxin, and through secreting hydrolytic enzymes. *P. polymyxa* M-1 is capable of colonizing wheat roots. By fluorescence *in situ* hybridization (FISH), confocal laser scanning microscopy (CLSM) and electron microscopy (EM), it was found that wheat colonization by M-1 was restricted to the rhizoplane. Few bacteria were observed in the internal tissues of wheat roots inoculated with M-1 in advance. M-1 colonized preferentially at the junction of primary roots and lateral roots, the junction of primary roots and root hairs as well as root hair surface by forming biofilms consisting of extracellular matrix and cells.

CS05-6

Loss of virulence in the phytopathogen *Ralstonia solanacearum* through infection by φRSM filamentous phages

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Ralstonia solanacearum is a widely distributed soil-borne phytopathogen belonging to the βsubdivision of Proteobacteria. It causes lethal bacterial wilt of more than 200 plant species, including economically important crops. During infection, *R. solanacearum* cells express various virulence and pathogenicity factors resulting in typical wilting symptoms in host plants. φRSM1 and φRSM3 (φRSM phages) are filamentous phages (inoviruses) that infect *R. solanacearum*. Infection by φRSM phages causes several cultural and physiological changes to host cells, especially loss of virulence. In this study, we characterized changes related to the virulence in φRSM3-infected cells, including (i) reduced twitching motility and reduced amounts of type IV pili (Tfp), (ii) lower levels of β-1,4-endoglucanase (Egl) activity and extracellular polysaccharides (EPS) production, and (iii) reduced expression of certain genes (egl, pehC, phcA, phcB, pilT, and hrpB). The significantly lower levels of phcA and phcB expression in φRSM3-infected cells suggested that functional PhcA was insufficient to activate many virulence genes. Tomato plants injected with φRSM3-infected cells of different *R. solanacearum* strains did not show wilting symptoms. The virulence and virulence factors were restored when φRSM3-encoded orf15, the gene for a putative repressor-like protein was disrupted. Expression levels of phcA as well as other virulence-related genes in φRSM3-dORF15-infected cells were comparable with those in wild type cells, suggesting that orf15 of φRSM3 may repress phcA and consequently result in loss of virulence. Similar effects on loss of virulence were also observed for infection with another group of filamentous phages (φRSS phages) in *R. solanacearum*.

CS06-1

Finding new candidate parasitism genes in plant parasitic nematodes: an evolutionary and comparative genomics approach

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The Southern root-knot nematode (RKN) *Meloidogyne incognita* is a mitotic parthenogenetic parasite able to infect the roots of almost all cultivated plants, which possibly renders this species the most damaging crop pathogen in the world. We have deciphered the genome of this nematode species which represented the first whole genome sequence assembly and annotation for a plant-parasitic nematode. As part of this genome annotation we first identified a set of RKN-specific genes, based on a comparative analysis with 25 eukaryotic genomes. We showed that more than half of the predicted proteins in *M. incognita* could not be assigned an ortholog in any of the eukaryotic genomes considered based on reciprocal best blast hits criteria (OrthoMCL). Because our goal is to identify druggable parasitism genes, we discarded all genes that had a predicted ortholog with OrthoMCL or that showed significant similarity in Blast analysis with species that could represent co-lateral damage (e.g. plants, chordates, pollinator insects).Using a series of bioinformatic screens, we selected nematode genes which were further analyzed with design of siRNAs and infestation test experiments after silencing. In total 10 out of the 15 inactivated genes showed a significant reduction in the number of egg masses or gall numbers compared to the control. Reductions in the number of egg masses or galls reached as much as 60% compared to the control. Such a protein set could therefore represent a putative wealth for identifying specific targets to develop sharp strategies against these pests.

CS06-2

Mining the active proteome of nematode-induced feeding cells in roots of *Arabidopsis thaliana*

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The cyst nematode *Heterodera schachtii* infects roots of Arabidopsis and parasitizes by modifying root cells to a hypertrophic syncytial feeding cell system. Nematodes secrete effectors that manipulates host protein activities in a network of interactions by post-translational modifications e.g. inhibition and activation. Transcriptomic and proteomic approaches cannot display this functional proteomic information. Activity-based protein profiling (ABPP) is a method to investigate the activity of proteome using activity based probes (ABPs). ABPs are molecular probes that react with a subset of enzymes in an activity dependent manner. In this way, all those proteins are ruled out, which are not activated. We applied ABPP using three different probes (MV151, FP, MV101) to display differential enzyme activities in syncytium induced by *H. schachtii*. Our analysis shows that the activity of several groups of enzymes is differentially regulated in syncytium. Among those specifically suppressed in syncytium are proteasomal subunits (β1, β2, β5), several Papain-like cysteine proteases (PLCPs i.e. Cathepsin, RD21, AALP, XCP etc.) and vacuolar processing enzymes (VPEs). An analysis of transcriptional data for proteasomal subunits revealed an accumulation of transcripts in syncytium. These results imply suppression of proteasome activity in syncytium. Similarly, activity of a serine carboxypeptidase-like protein (SCPL), a S-formyl-glutathione hydrolase (SFGH) and methylesterase is specifically up-regulated in syncytium. We characterized the role of some of these differentially regulated enzymes (Cathepsin, VPEs, RD21, AALP, XCP) by using T-DNA insertion knock-out mutants. This analysis revealed a change in susceptibility of plants to nematodes. Our analysis provides a first insight into functional proteomics of syncytium.

CS06-3

Interaction between root-knot nematodes and plant signaling networks during parasitic invasion

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Root-knot nematodes (RKNs) are obligate parasites that attack plant roots. Following invasion of root tips as a juvenile, they establish a specialised feeding site next to the vascular tissue and remain protected at this single site for their whole life-cycle. RKNs do not show high host specificity and are able to parasitise a broad range of important plant species. Understanding of RKN parasitism at the molecular level is currently limited, particularly in the context of the broad host range. It was generally thought that RKNs avoid activating the host defense response during the initial infection stages. However, we have now shown that invasion by *Meloidogyne hapla* RKN is indeed recognised by the host and elicits strong defense-like signaling in root tissues. The fact that this local signaling does not develop into an effective immune response raised the possibility that RKNs manipulate an additional host signaling network to gain acceptance of infection site formation. Using a series of genetic mutants, we now show that a defect in a common plant signaling pathway limits the parasitic ability of *M. hapla*. RKN development stalled in the mutant roots soon after initiation of infection, consistent with a direct role for the signaling pathway in infection site development rather than an indirect effect related to root entry or target cell selection. These data reveal a common network used by *M. hapla* to achieve parasitic success that can also explain its compatibility with different host plants.

CS06-4

Tritrophic interactions among thrips, tospovirus and Arabidopsis

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The western flower thrips (*Frankliniella occidentalis*) is a polyphagous herbivore that causes serious damages on many agricultural plants and also transmits tospoviruses, such as *Tomato spotted wilt virus* (TSWV). Therefore, feeding damage and virus disease caused by thrips attack are serious problems in many countries. Our previous study reported that JA plays an important role to plant response and resistance to thrips, and JA-regulated plant defense decrease thrips performance and preference. In this meeting, we report the analyses of the tritrophic interaction between plants (Arabidopsis plants) and insect vector (western flower thrips), and also plant virus (TSWV). In ecological system, TSWV only move to the new host plants from the infected host plants by insect vector, thrips. We indicate that TSWV infection enhances thrips performance such as feeding activity and increases thrips population density of next generation in Arabidopsis plants. TSWV infection elevated Salicylic acid (SA) contents and induced SA-

regulated gene expression. Meanwhile, TSWV infection decreased thrips feeding inducible JA-regulated gene expression. We also indicate that TSWV infection enhances the thrips preference of host plants. Thrips was attracted to the TSWV infected plants, and its attractance was decreased in JA insensitive *coi1-1* mutants as compared to WT plants. In addition, SA application to WT plants enhanced this thrips attractance like TSWV infection. Our results suggest the mechanism of virus strategy to attract vector thrips to virus-infected plants taking advantage of antagonistic SA-JA plant defense system.

CS06-5

Rewiring of the jasmonate signaling pathway in Arabidopsis during insect herbivory

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Plant defenses against insect herbivores and necrotrophic pathogens are differentially regulated by different components of the jasmonic acid (JA) signaling pathway. In Arabidopsis, the basic helix-loop-helix leucine zipper transcription factor MYC2 and the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) domain transcription factor ORA59 antagonistically control distinct branches of the JA pathway. Feeding by larvae of the specialist insect herbivore *Pieris rapae* activated *MYC2* transcription, which led to expression of the MYC-branch marker gene *VSP2* and suppression of the ERF-branch regulator *ORA59* and the ERF-branch marker gene *PDF1.2* (Verhage et al., 2011; Frontiers Plant Sci. 2:47). The hormone abscisic acid (ABA) was identified as a critical component in rewiring of the MYC- and ERF-branches of the JA pathway during insect herbivory. In two-choice setups with mutant and wild-type plants, the larvae consistently preferred to feed on plants that had activated the ERF-branch over plants that had activated the MYC-branch or neither one of the two branches. This suggests that the herbivores were stimulated to feed from plants expressing the ERF-branch rather than that they were deterred by plants expressing the MYC-branch. Interestingly, application of larval oral secretion into wounded leaf tissue stimulated the ERF-branch of the JA pathway, suggesting that compounds in the oral secretion have the potential to manipulate the plant response toward the caterpillar-preferred ERF-regulated branch of the JA response. Our results suggest that by activating the MYC-branch of the JA pathway, plants prevent stimulation of the ERF-branch by the herbivore, thereby becoming less attractive to the attacker.

CS06-6

Involvement of MAP kinase cascade and NO in plant immune response to *Henosepilachna vigintioctopunctata*

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Henosepilachna vigintioctopunctata is a herbivorous insect that feeds solanaceae crops. Emerging evidence emphasizes that plants have developed defense mechanisms to insects as well as pathogens. However, the detailed mechanisms are unclear. To investigate factors involved in herbivorous insect resistance of solanaceae plants, we developed a model system using *H. vigintioctopunctata* and *Nicotiana benthamiana*. The model system is useful for analysis of herbivorous insect - plant interactions, because RNAi technology is available for both *H. vigintioctopunctata* and *N. benthamiana*. Virus-mediated silencing of *SIPK* and *WIPK*, which are MAP kinases involved in immune response to various pathogens, decreased resistance to *H. vigintioctopunctata*. Furthermore, SIPK and WIPK proteins are phosphorylated by *H. vigintioctopunctata* feeding. We have reported that MAPK signaling regulates NO and RBOH-dependent ROS bursts in *N. benthamiana*. Silencing *NbRBOHB* did not affect insect resistance, whereas treatment of L-NAME, which is an NO synthase inhibitor, decreased resistance to *H. vigintioctopunctata*. In addition, NO production was induced by *H. vigintioctopunctata* feeding. Thus, these results suggest that MAPK cascades and NO have important roles in plant immune responses to *H. vigintioctopunctata*.

CS07-1

***Pseudomonas syringae* type III effectors**

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Like many pathogens, *P. syringae* harbors dozens of effectors that are potentially injected into host cells to promote bacterial fitness and/or cause disease. A decade has passed since our first global analysis of effector repertoires. In this talk, I will discuss our recent progress in understanding the roles of effectors in specific niches, their biochemical activities and the value of continuing to study effectors using multiple approaches.

CS07-2

The *Xanthomonas oryzae* pv. *oryzae* type III effector XopR alters ethylene perception and signal transduction pathway post MAMPs treatment, suppresses plant innate immunity in *Arabidopsis thaliana*

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Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal agent of bacterial blight of rice. The XopR protein, secreted into plant cells through the type III secretion apparatus, is widely conserved in xanthomonads and is predicted to play important roles in bacterial pathogenicity. We have reported that XopR inhibited basal defense responses in plants rapidly after MAMP recognition (MPMI 25:505-514 2012). To address XopR function in plant, early events occurred by flg22 treatment on XopR transgenic *Arabidopsis* plants were tested. MAP kinase activation was not changed in XopR-expressing plant. On the contrast, oxidative burst triggered by flg22 was abolished under the XopR-expressing condition. Similar phenotypes were reported on flg22-treated *etr1-1* and *ein2-1* mutants both defective in ethylene perception (Plant Physiol. 154:391-400 2010), the triple response on etiolated seedling, typical ethylene response test, was observed. In XopR expressing plants, apical hook curve was weakened under ethylene treatment, in addition stem elongation was diminished under silver ion (ethylene perception inhibitor) treatment. Oxidative burst triggered by elf-18 and chitin oligomer on XopR-expressing plant were abolished as in case of flg-22 treatment. Taken together, it was suggested that XopR attacks ethylene perception and common signal transduction pathway post MAMPs perception not particular MAMP receptor.

CS07-3

The *Pseudomonas syringae* type III effector HopD1 targets the ER-localized Arabidopsis transcription factor NTL9 and blocks effector-triggered immunity

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The phytopathogenic bacteria *Pseudomonas syringae* injects effector proteins into plant cells via the type III secretion system in order to suppress host immunity. Here we show that one of these effectors, HopD1, contributes to virulence, is a strong suppressor of effector-triggered immunity, and localizes to the endoplasmic reticulum (ER) of plant cells. Protein-protein interaction assays identify the Arabidopsis transcription factor NTL9 as a target of HopD1. NTL9 is a membrane bound transcription factor that due to a C-terminal transmembrane domain resides in the ER. Upon activation, the transmembrane domain of NTL9 is removed by proteolytic cleavage and NTL9 enters the nucleus where it induces gene transcription. We hypothesize that HopD1 promotes virulence of *P. syringae* by targeting NTL9 to prevent its activation or release from the ER and thus the transcription of genes associated with effector-triggered immunity.

CS07-4

Toward understanding *Magnaporthe oryzae* effector functions

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Rice blast caused by the ascomycete fungus *Magnaporthe oryzae* is the most devastating disease of rice worldwide, therefore understanding of the molecular mechanisms of *Magnaporthe*-rice interactions is important to devise efficient control of the disease. Using *M. oryzae* whole genome sequence information and association genetics approach, we isolated genes for three AVRs, AVR-Pia, AVR-Pii and AVR-Pik/km/kp as well as other effector candidates. All three AVRs were shown to be delivered to rice cells. Using biochemical approaches, we are trying to elucidate their effector functions. In this paper, I show our latest findings on their interactions with rice factors including R-proteins.

CS07-5

High resolution crystal structure of *Cladosporium fulvum* LysM effector Ecp6

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Plants induce immune responses upon pathogen attack due to their ability to recognize microbial-derived molecular components, such as fungal cell wall chitin. To prevent this induction of immune responses, fungal plant pathogens secrete large amounts of LysM effectors which sequester chitin oligossaccharides preventing their recognition and the induction of host defence response (1-3). Lysin motifs (LysM) are highly conserved domains present in several prokaryotic and eukaryotic proteins which are known to bind various carbohydrates, including peptidoglycan and chitin. Despite the biological relevance of LysM domain-containing proteins, the biochemistry of the interaction with their substrates has not yet been elucidated. In this work, we present a model of the molecular mechanisms of the *Cladosporium fulvum* LysM effector ECP6 based on its high-resolution crystal structure. The structure provides evidence for a high-affinity binding pocket within the LysM domains to which chitin oligomers are directly bound and captured to avoid their recognition by plant receptors. Biochemical and biological data will be presented and the molecular mechanism of LysM effectors function will be discussed.
(1) de Jonge R. et al. (2010); (2) Marshall R. et al. (2011); (3) Mentlak T. A. et al. (2012).

CS07-6

Effectors secreted by plant pathogenic oomycetes as molecular probes to understand focal immune responses at pathogen penetration sites

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Oomycete pathogens such as *Phytophthora infestans* form accommodation structures termed haustoria to deliver pathogenicity effector proteins and acquire nutrients. The haustorium is surrounded by a host-derived membrane called extrahaustorial membrane (EHM), which differs from plasma membrane in various aspects. However, the composition of the EHM and the mechanisms underlining its biogenesis are poorly understood. We recently showed that plasma membrane localized proteins are selectively excluded from EHM, whereas some plasma membrane associated proteins and proteins mediating vesicle trafficking localized around the EHM. In addition, we recently discovered that a host-translocated RXLR-type effector protein AVRblb2 of *P. infestans* focally accumulates at the EHM, while another RXLR effector HaRXL17 secreted by *Hyaloperonospora arabidopsidis* localizes to the tonoplast surrounding the EHM in *N. benthamiana* infected by *P. infestans*. We hypothesized that AVRblb2 and HaRXL17 can be used as molecular probes to better understand the composition of the EHM and to gain insights about mechanisms for its biogenesis. For this, we co-expressed the effectors with plant proteins that localize around the EHM in infected cells, but label different subcellular compartments in uninfected cells and/or implicated in plant microbe interactions. These experiments revealed that: (i) a trafficking pathway between vacuole or pre-vacuoler compartments and EHM; (ii) rather than being uniform the EHM appears as a patchwork highlighted by different marker proteins; and (iii) some host proteins might localize at the EHM in a spatio-temporal manner. Our findings indicate that effectors such as AVRblb2 are unique tools to understand focal responses at pathogen penetration sites.

CS08-1

Microtubule (+)-end-associated protein interacting with potyviral helper component proteinase

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Potato virus A (PVA) belongs to genus *Potyvirus* that is the largest and economically the most important group of plant-infecting RNA viruses. Potyviral helper component proteinase (HCpro) is a multifunctional protein involved e.g. in virus amplification, movement and suppression of the antiviral defence mechanism, RNA-silencing. HCpro of PVA interacts with potato protein HIP2. Our results show that HIP2 is an ortholog of microtubule-associated *Arabidopsis* proteins SPR2 and SP2L. Transiently expressed HIP2 tagged with red fluorescence protein localized to microtubules *in planta*. The interacting HCpro and HIP2 also localized to cortical microtubules during virus infection, as analysed using bimolecular fluorescence complementation (BiFC). The C-proximal alpha helix rich domain of HIP2 controlled HIP2-HCpro interaction, whereas the N-proximal TOG and coiled-coil domains of HIP2 controlled dimerization and binding of HIP2 to microtubules. Silencing of *HIP2* in *Nicotiana benthamiana* resulted in a spiral-like growth phenotype, similar to *Arabidopsis spr2* mutant, and the *spr2* phenotype in Arabidopsis was complemented with potato HIP2. Accumulation of PVA was significantly reduced in the HIP2-silenced leaves of *N. benthamiana*, indicating that HIP2-HCpro interactions are important for virus infection. Movement of PVA was not altered significantly.

CS08-2

***Tobacco mosaic virus* movement protein co-targets to plasmodesmata with virus-induced host β-1,3-glucanases**

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Cell-to-cell spread of plant viruses is dependent on virus encoded movement proteins (MPs) that target to and gate plasmodesmata (Pd). We recently showed that replication of *Tobacco mosaic virus* (TMV) in the absence of its MP results in increased callose accumulation at Pd and decreased Pd permeability, while in the presence of MP the accumulation of callose is reduced and Pd permeability increased (Guenoune-Gelbart, et al., 2008. Mol Plant Microbe Interact 21:335-45). It was suggested that TMV replication induces host β-1,3-glucanases (BGs), which are targeted by MP to Pd thus reducing callose accumulation and facilitating viral spread. We show here that infection of Arabidopsis with various viruses induces expression of two pathogenesis related BG proteins, AtBG2 and AtBG3. When AtBG2 is expressed in *Nicotiana benthamiana*, it accumulates in ER strands that transect walls at Pd. It is not exported to the wall. In transgenic *N. benthamiana* that over-expressing AtBG2-GFP and which were infected with TMVδCP MP-RFP, AtBG2-GFP co-localizes with MP-RFP in ER-derived bodies which target to Pd sites. Other ER resident luminal and membrane proteins also co-localize with MP-RFP in bodies that are appressed to Pd sites at the infection front. As the virus spreads to adjacent cells ER-derived bodies are first formed in the newly infected cell on the wall contiguous to the source of infection. Data is presented which suggest that BG2 is redundant and that multiple factors may be involved in virus spread through the Pd.

CS08-3

Replication-independent long-distance trafficking of *Bamboo mosaic virus* satellite RNA

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Satellite RNAs, the subviral agents, completely depend on their helper viruses for replication and encapsidation. However, how satellite RNAs traffic in the whole plants is largely unknown. Previously, we showed that *Bamboo mosaic virus* satellite RNA (satBaMV) is dependent on BaMV for efficient long-distance trafficking in *Nicotiana benthamiana* plants. The satBaMV-encoded P20 protein is an RNA binding protein that facilitates the systemic movement of satBaMV in the co-infected plants. Here, we demonstrated that the systemic movement of P20-defective satBaMV can be transcomplemented in transgenic *N. benthamiana* expressing P20 protein. To examine if satBaMV can traffic alone, the scions of wild-type plants were grafted onto the rootstocks of transgenic *N. benthamiana* expressing the full-length cDNA of satBaMV and vice versa. The satBaMV RNA could be detected in the scions or rootstocks of wild-type plants by northern blot analysis 3-6 days post grafting. Deep sequencing of small RNAs from scion stems revealed that satBaMV-specific siRNA preferentially mapped to P20 region of satBaMV. No satBaMV RNA or siRNA was detectable in the wild-type controls. Moreover, fibrillarin was co-immunoprecipitated with P20 protein when total proteins from BaMV and satBaMV co-infected leaves of *N. benthamiana* were treated with anti-P20 serum. By virus-induced gene silencing, the fibrillarin-silenced plants strongly suppressed the long-distance trafficking of satBaMV. Taken together, these results suggest that host fibrillarin plays a vital role in satBaMV long-distance trafficking by replication-independent manner.

CS08-4

Transgene viral siRNA profile and its effect on cucurbit viral resistance

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Viral resistance based on gene silencing has been developed for many viruses. However, little is known concerning the transgene-small-interfering RNA (t-siRNA) population causing viral resistance. Transgenic cucumber and melon plants were constructed bearing a hairpin construct including a fragment of the *Zucchini yellow mosaic potyvirus* (ZYMV) HC-Pro gene. Transgenic lines accumulating t-siRNA exhibited resistance to systemic ZYMV infection. In resistant lines t-siRNA comprised 12-44% of total small RNA in cucumber and 6-8% in melon, determined by Illumina sequencing. The majority of t-siRNA in transgenic melon and cucumber was 21 (40-60%) and 22 nts (28-35%), while accumulation of 24 nts t-siRNA (20%) was found only in a cucumber line harboring high t-siRNA levels. Uneven t-siRNA densities along the transgene sequence were characterized, reflecting accumulation of t-siRNA in hot spots. One transgenic line exhibited resistance to systemic infection of four different RNA viruses, independent of homology between the transgene sequence and the virus. This line accumulated an exceptionally high level of t-siRNA, 43% of total plant siRNA, in addition to increased level of RNA-dependent-RNA-polymerase 1 (RDR1). Our data show for the first time a correlation between a broad RNA virus resistance and an increased level of RDR1 mRNA expression. We suggest a new model in which a high level of t-siRNA increases RDR1 expression leading to the induction of broad viral resistance, independent of involvement of salicylic acid.

CS08-5

The single dominant resistance gene *Tsw* is triggered by a functional RNA silencing suppressor protein of the *Tomato spotted wilt virus*

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The dominant resistance gene *Tsw* in *Capsicum annuum* against the *Tomato spotted wilt virus* (TSWV) has been broken in the field by various isolates of this virus. To determine the identity of the avirulence-protein, the N and NSs genes of resistance inducing (RI) and resistance breaking (RB) isolates were cloned and transiently expressed in resistant *Capsicum* plants. It is shown that the NSs, the TSWV RNA silencing suppressor (RSS) protein, of the RI isolate triggers an Hypersensitive Response (HR) in *Tsw* containing *Capsicum* plants while no HR was discerned after expression of the N protein, or when NSs from an RB isolate was expressed. Whereas NSs from the RI isolate was able to suppress the silencing of a functional GFP construct during *Agrobacterium tumefaciens* transient transformation assays on *Nicotiana benthamiana* plants, NSs from the RB isolate had lost this capacity. Surprisingly, local GFP silencing could still be suppressed when a co-infection with RB or RI viruses was performed. Earlier, the NSs protein was shown to exert RSS activity by sequestering small (si- and mi-) RNAs. Electrophoretic mobility shift assays revealed that the NSs protein of RB isolates, in contrast to the NSs from RI isolates, lost their affinity to short-interfering (si)RNAs. Altogether these data demonstrate that NSs triggers *Tsw*-mediated resistance and suggest a putative link between the mechanism of dominant virus resistance and RNA silencing. Currently, alanine substitution analysis is being performed to map domains within the NSs protein involved in RSS activity and/or triggering of HR.

CS08-6

Genetic, biochemical, and structural studies about interactions between *Tomato mosaic virus* and the resistance gene *Tm-1*

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The tomato *Tm-1* gene is a resistance gene against *Tomato mosaic virus* (ToMV). *Tm-1* was introgressed from a wild tomato species *Solanum habrochaites* and encodes a protein that inhibits ToMV RNA replication through binding to the replication proteins. Homologous genes to *Tm-1* are widely conserved not only among plants but also even in fungi, bacteria, and archaea, suggesting that *Tm-1* has a primary function other than ToMV resistance and incidentally acquired the ability to bind ToMV replication proteins. To examine whether natural selection has acted on *Tm-1* for the evolution of ToMV resistance, we analyzed the *Tm-1* alleles of *S. habrochaites*. Whereas most parts of the gene were under purifying selection, a small region (approx. 30 aa) showed a signature of positive selection. Deletion analysis of the Tm-1 protein suggested that functional fragments for the inhibition of ToMV RNA replication contained the positively selected region. Unexpectedly, we found *Tm-1* alleles from *S. habrochaites* that inhibit RNA replication of LT1, a *Tm-1*-resistance-breaking ToMV mutant. An amino acid change in the region under positive selection was identified to be responsible for the ability to inhibit LT1 multiplication. Biochemical analyses suggested that the amino acid change makes Tm-1 a more potent inhibitor and capable of binding to LT1 replication proteins which does not bind the original Tm-1. Finally, we will discuss the interaction between Tm-1 and ToMV replication proteins from a structural view.

CS09-1

Oligogalacturonides alert the plant immune system to cell wall damage

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Activation of the immune system in animals and plants by endogenous molecules released upon tissue injury (damage-associated molecular patterns or DAMPs) is emerging as an essential part of the strategies evolved for survival. Extracellular DAMPs, like microbe-associated molecular patterns (MAMPs), are recognized by plasma membrane receptors indicated as Pattern Recognition Receptors (PRRs). Oligogalacturonides (OGs), released from the homogalacturonan of the cell wall upon mechanical damage or upon digestion by pectinases secreted by invading pathogens, are the best characterized class of plant DAMPs. An overlap exists between the defense responses activated in Arabidopsis by OGs and those activated by the bacterial MAMPs flg22 and elf18. Both type of elicitors activate a signal transduction pathway that involves phosphorylation of the *Arabidopsis thaliana* MAP kinases MPK3 and MPK6, and are capable of repressing responses induced by auxin. We have identified novel elements of the MAP kinase cascade that mediate activation of defense responses by OGs. Dynamics studies *in vivo* reveal a function of these kinases in the regulation of elicitor-induced ROS production by mitochondria and plastids.

CS09-2

Uncoupling resistance to pathogens from tradeoffs by remodeling Arabidopsis cell wall

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The understanding of the dynamics and evolution of plant defensive responses is of fundamental importance as they impact agricultural yield. In particular, the characterization of trade-offs associated to broad-spectrum, durable resistance in crops needs further attention. The contribution of plant cell wall to this type of resistance was analysed in *Arabidopsis thaliana* by determining the susceptibility/resistance of 120 cell wall mutants (*cmw*) to different types of pathogens. We identified a significant number of *cmw* mutants that uncoupled resistance to pathogens from yield, further indicating that wall remodelling might be an efficient strategy to overcome trade-offs associated to pathogen resistance. The relevance of cell wall-mediated resistance to pathogens was also supported by the finding that key components of *Arabidopsis thaliana* defensive mechanisms, such as the ERECTA (ER) Receptor Protein Kinase, the ELK2 Mitogen-Activated Protein Kinase Kinase Kinase (MAP3K), and the β subunit of the heterotrimeric G protein (AGB1) modulated the immune response by regulating cell wall integrity. Mutants impaired in these genes showed a mis-regulation of cell wall-associated genes and alterations in cell wall composition/structure compared with those of wild-type plants. The characterization of the genetic and molecular bases of the resistance in these mutants revealed that novel, previously uncharacterised signalling pathways controlled their defensive responses. Moreover, we found that Arabidopsis immune response can be modulated by cell wall signals (DAMPs, damaged-associated molecular patterns) derived from pathogen-resistant *cwm* plants. All these data suggest that remodelling of cell wall would be an efficient strategy in pathogen-resistance breeding programs.

CS09-3

Cell wall acetylation plays a pivotal role in the cuticle assembly and susceptibility to nectrotic fungal pathogen *Botrytis cinerea*

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Here we show that the cell wall acetylation plays an important role in pathogen susceptibility in *Arabidopsis thaliana*. We have recently identified REDUCED WALL ACETYLATION 2, which is required for the full acetylation of the cell wall polysaccharides (1). The mutants showed an increased resistant to *Botrytis*, indicating that cell wall acetylation is a susceptibility factor. In the present study the leaves of *rwa2* mutants (*rwa2-1* and *rwa2-3*) were shown to exhibit an increased permeability to toluidine blue and callose and an enhanced rate of water loss as compared to those of WT. The majority of trichomes were collapsed or dead. Ultrastructural analysis revealed that the thickness of the cell wall increased by 30-40% of the WT levels in the mutant leaves with electron-dense materials, which is indicative of lipidic compounds, trapped in the cell wall of the mutants but not in that of WT. Application of chitosan to the seedling and *Botryis* to mature leaves induced enhanced callose and hydrogen peroxide depositions, respectively, in the mutants as compared to WT. These results indicate that the delivery and/or assembly of cuticular components through the cell wall is impaired in the mutants, leading to the increased water loss and the faster sensing of elicitors. Our results demonstrate that the cell wall acetylation plays a pivotal role in the surface assembly and suggest that acetylation is likely to have evolved to optimize the surface structures at the expense of pathogen resistance.
(1) Manabe et al. (2011) Plant Physiol 155:1068.

CS09-4

Poly(ADP-ribosyl)ation plays an essential role in pathogen-induced cell wall reinforcement

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Poly(ADP-ribosyl)ation is an important post-translational modification in which chains of poly(ADP-ribose) are added to a target protein. Although poly(ADP-ribosyl)ation has been studied deeply in mammalian systems due to its role in DNA damage repair and cell stress, novel roles for poly(ADP-ribosyl)ation in the plant innate immune response are being elucidated. Chemical inhibitors of poly(ADP-ribosyl)ation such as 3-amino-benzamide (3AB) block certain aspects of the plant innate immune response. Early steps after MAMP perception such as the production of reactive oxygen species remain intact, but some later steps, including cell wall reinforcement with callose depositions, are blocked. Although MAMP-induced callose is blocked, wound-induced callose is still deposited in the presence of 3AB despite the fact that the GSL5/PMR4 callose synthase enzyme is responsible for both. Quantitative RT-PCR analysis revealed that expression of *GSL5* increases approximately three-fold between two and four hours after MAMP (flg22) treatment. A similar increase in expression is observed when seedlings are treated with flg22 and 3AB, suggesting that 3AB does not block *GSL5* expression. Analysis of callose in *parp1*, *parp2*,

and *parp3* mutants revealed a reduction in flg22-induced callose in *parp3* mutants, while callose in *parp1* and *parp2* mutants appeared similar to wild type levels. A screen of Arabidopsis T-DNA mutants disrupted in genes whose MAMP-responsive expression is altered by poly(ADP-ribosyl)ation revealed two genes that impact callose deposition. These and other results are suggesting an important role for poly(ADP-ribosyl)ation in the plant innate immune response, and in pathogen-induced cell wall reinforcement in particular.

CS09-5

Reduced carbohydrate availability and altered pectin composition in Arabidopsis enhance susceptibility towards *Colletotrichum higginsianum*

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Colletotrichum higginsianum is a hemibiotrophic ascomycete fungus that is adapted to *Arabidopsis thaliana*. After breaching the host surface, the fungus establishes an initial biotrophic phase in the penetrated epidermis cell, before necrotrophic growth is initiated upon further host colonization. We could observe that Arabidopsis mutants with impaired starch turnover were more susceptible towards *C. higginsianum* infection, with starch-free mutants exhibiting the strongest susceptibility. By altering the length of the light phase and by employing additional genotypes impaired in nocturnal carbon mobilization, we could reveal that periodic nocturnal starvation for carbon represents an enhanced susceptibility factor in the investigated pathosystem. Especially, dark-induced starvation during the necrotrophic phase increased the susceptibility of the host. Most importantly, systematic starvation experiments could reveal that nutrient supply by the host is dispensable during the biotrophic phase of *C. higginsisanum*. However, early post-penetration establishment of the fungus was also most strongly increased in starchless mutants. An in-depth analysis of cell wall composition and the comparison to described cell wall mutants demonstrated that pectin of the starch-free mutants contained less arabinose, galactose and galcturonic acid residues, which could be attributed to enhanced establishment of *C. higginsianum*. In contrast, the starchless mutants were more resistant towards the fungal biotroph *Erysiphe cruciferarum*, which was not due to the observed changes in pectin composition, indicating that the two identified susceptibility factors, periodic carbon shortage and pectin composition, do not increase susceptibility in all interactions.

CS09-6

Links between the cell wall and powdery mildew disease resistance in Arabidopsis

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The *pmr5 (powdery mildew resistant 5)* mutant was found in a screen for genes involved in susceptibility to *Golovinomyces cichoracearum*, a biotrophic pathogen that infects Arabidopsis. PMR5 is a member of the TBL (TRICHOME BIREFRINGENCE LIKE) family, which is composed of 46 functionally uncharacterized plant-specific proteins. Initial characterization of this mutant showed that *pmr5*-mediated disease resistance acts independently of the salicylic acid, jasmonic acid, and ethylene signal transduction pathways, and that there are changes in the *pmr5* cell wall that may be linked to the gain of resistance in the mutant (Vogel *et al*., 2004). We characterized the cell wall composition in more detail and believe PMR5 affects pectin, specifically rhamnogalacturonan I. Several other cell wall mutants have shown enhanced resistance to plant pathogens, leading to the hypothesis that there may be a novel cell wall integrity signaling pathway that is triggering downstream defense responses (Hematy *et al.,* 2009). We hypothesize that the cell wall changes in *pmr5* are activating a constitutive defense response against the host powdery mildew, and aim to characterize this novel defense pathway by microarray analysis and suppressor mutant characterization.

CS10-1

Roles of CBP60 proteins in the plant defense network

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Calmodulin Binding Protein 60 (CBP60) proteins comprise a small family of eight members in Arabidopsis. Plants with *cbp60g* loss-of-function mutations are more susceptible to *Pseudomonas syringae* pv. *maculicola* strain ES4326 (*Pma* ES4326) and have reduced levels of salicylic acid (SA) at early times after infection. CBP60g binds calmodulin (CaM) through a CaM-binding domain at the amino-terminus. CaM binding is required for function, as mutants that cannot bind CaM do not complement the pathogen growth or SA phenotypes. The family member most closely related to CBP60g is called SARD1. Plants with *sard1* loss-of-function mutations are also more susceptible to *Pma* ES4326, and have reduced levels of SA at later times after infection. SARD1 does not bind CaM. Plants doubly mutant for *cbp60g* and *sard1* display severe enhanced susceptibility to *Pma* ES4326 and greatly reduced SA levels. Expression profiling experiments demonstrated that CBP60g and SARD1 are required for expression of SA-dependent genes, as well as another group of genes that are SA-independent but dependent on PAD4 and EDS1. A clustering analysis of genes co-expressed with genes known to have reduced expression in *cbp60g sard1* plants identified additional genes whose expression is affected by CBP60g and SARD1. Curiously, some of these show reduced expression in *cbp60g sard1* plants, while others show increased expression. The family member most closely related to CBP60g and SARD1 is CBP60a. Plants with *cbp60a* loss-of-function mutations are more resistant to *Pma* ES4326 than wild-type plants, and have elevated SA levels in the absence of infection.

CS10-2

Dynamic regulation of plant immune response

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Plants are sessile organisms; therefore, their physiological programs are highly entrained with environmental cues, resulting in circadian rhythms and seasonal life cycles. Since plant cells are polypotent, their responses to the environmental cues have to be balanced with growth and development. This balanced is maintained through sophisticated regulatory mechanisms. In my talk, I will present evidence for the circadian clock regulation of plant defense in anticipation of infection at the time of the day when the pathogen threat is the highest. Under pathogen challenge, infected cells can undergo programmed cell death (PCD) to restrict pathogen growth, whereas the intact cells need to turn on anti-PCD genes to prevent the spread of cell death and activate systemic acquired resistance. I will show data on how cell death and survival is regulated through perception of the plant immune signal, salicylic acid, which controls the function of the key immune regulator, NPR1. Moreover, we also discovered a synergism between plant immune responses and DNA damage repair mechanism. Through genetic screens and subsequent characterization, we found that genes involved in damage repair are directly associated with transcription of plant immunity-related genes. The potential significance of this synergy between these two most fundamental stress responses will be discussed.

CS10-3

Rice WRKY45 plays a key role in priming of diterpenoid phytoalexin biosynthesis through the salicylic acid signaling pathway

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Plant activators such as benzothiadiazole (BTH) prime defense responses in plants by acting on the salicylic acid (SA) signaling pathway. In rice, BTH-inducible transcription factor WRKY45 plays a key role in BTH-induced disease resistance through the rice SA pathway, which branches into OsNPR1/NH1- and WRKY45-dependent subpathways. Overexpression of *WRKY45* (*WRKY45*-ox) conferred strong resistance against fungal blast (*Magnaporthe oryzae*) to rice due to pre-invasive defense. To elucidate the mechanism underlying WRKY45-mediated blast resistance, we performed expressional analysis focusing on diterpenoid phytoalexin (DPA) biosynthesis genes. Microarray analysis revealed that the expression of DPA biosynthesis genes was only moderately upregulated in *WRKY45*-ox rice, but the expression levels increased rapidly after *M. oryzae* inoculation compared with non-transformant rice plants. The gene expression pattern was reflected in the accumulation of DPAs, momilactones and phytocassanes. In non-transformant rice plants, either BTH treatment or *M. oryzae* inoculation alone barely upregulated the DPA biosynthesis genes, but they were highly upregulated when *M. oryzae* was inoculated to BTH-treated rice plants at 1 day post-inoculation. The upregulation was not observed in *WRKY45*-knockdown rice plants. These results indicate that WRKY45 plays a role in priming of the induction of DPA biosynthesis genes by BTH. Cytokinin has been known to induce the expression of DPA biosynthesis genes in rice. In our system using rice leaf discs, cytokinin alone upregulated them only slightly, but co-treatment with SA and cytokinin dramatically upregulated them, suggesting a role of cytokinin in triggering defense gene expression in plants primed by SA/BTH.

CS10-4

Hormonal modulation of plant immunity

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Crosstalk between the defense hormones salicylic acid (SA) and jasmonic acid (JA) plays a central role in the modulation of induced plant immune responses (1). In Arabidopsis, the SA pathway antagonizes the JA signaling sector of the plant immune signaling network. We aim to unravel how SA exerts its antagonistic effect on the JA signaling sector. Here, we show that suppression of the JA pathway by SA functions downstream of the E3 ubiquitin-ligase SCF^{COI1} complex that targets JASMONATE ZIM-domain transcriptional repressor proteins (JAZs) for proteasome-mediated degradation. The JAZ proteins themselves were shown not to be a target for SA. Instead, the antagonistic effect of SA appears to be directly targeted at the level of gene transcription. In silico promoter analysis of the SA-JA crosstalk transcriptome of Arabidopsis revealed that the 1-kb promoter regions of JA-responsive genes that were suppressed by SA are significantly enriched in GCC-box motifs, which are binding sites for AP2/ERF transcription factors. Using plants carrying the *GUS* reporter gene under control of the GCC-box, we demonstrated that the GCC-box is a sufficient element for SA-induced suppression of JA-induced gene expression. We further provide evidence that SA stimulates degradation of the ERF-type transcription factor ORA59. Collectively, our data indicate that SA-mediated suppression of JA signaling is mediated by targeting the stability of positive transcriptional regulators of the JA response.
(1) Pieterse C. M. J., Van der Does, D., Leon-Reyes, A., and Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28: doi: 10.1146/annurev-cellbio-092910-154055.

CS10-5

Brassinosteroids antagonize gibberellin- and salicylate-mediated root immunity in rice

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Brassinosteroids (BRs) are a unique class of plant steroid hormones that orchestrate myriad growth and developmental processes. Although BRs have long been known to protect plants from a suite of biotic and abiotic stresses, our understanding of the underlying molecular mechanisms is still rudimentary. Aiming to further decipher the molecular logic of BR-modulated immunity, we have examined the dynamics and impact of BRs during infection of rice with the root oomycete *Pythium graminicola*. Challenging the prevailing view that BRs positively regulate plant innate immunity, we show that *P. graminicola* exploits BRs as virulence factors and hijacks the rice BR machinery to inflict disease. Moreover, we demonstrate that this immune-suppressive effect of BRs is due, at least in part, to negative crosstalk with salicylic acid (SA) and gibberellic acid (GA) pathways. BR-mediated suppression of SA defenses occurred downstream of SA biosynthesis, but upstream of the master defense regulators OsNPR1 and OsWRKY45. In contrast, BR alleviated GA-directed immune responses by interfering at multiple levels with GA metabolism, resulting in indirect stabilization of the DELLA protein and central GA repressor SLR1. Collectively, these data favor a model whereby P. graminicola co-opts the plant BR pathway as a decoy to antagonize effectual SA- and GA-mediated defenses. Our results highlight the importance of BRs in modulating plant immunity and uncover pathogen-mediated manipulation of plant steroid homeostasis as a core virulence strategy.

CS10-6

Tyrosine sulfated peptide receptors PSKR1 and PSY1R modulate *Arabidopsis* immunity

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Two tyrosine sulfated peptides PSKα and PSY1 have been shown to be bound by leucine-rich repeat receptors to control cell proliferation. Using a reverse genetics approach we identified the PSKα receptor, *PSKR1*, as an important component of plant immunity. *PSKR1* and a subset of genes encoding PSKα propeptides were transcriptionally up-regulated by pathogen treatment. *PSKR1* loss-of-function mutants were more resistant to the biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and had enhanced PAMP responses. Conversely, *PSKR1* mutants were more susceptible to the necrotrophic fungal pathogen *Alternaria brassicicola*, exhibiting increased lesion formation and fungal growth which is restricted in wild-type plants. These antagonistic defense responses were correlated with enhanced SA levels, enhanced *PR1* and *FRK1* expression and suppressed expression of *PDF1.2* and *OPR3* in *PSKR1* mutants. Analysis of multiple mutations in the paralogous receptors *PSKR1*, *PSKR2* and *PSY1R* revealed that PSKR1 and PSY1R, but not PSKR2, play an overlapping role in plant immunity. It was demonstrated that tyrosine sulfation by the tyrosine protein sulfotransferase TPST is critical for PSKα and PSY1 modification and signaling. *tpst-1* mutants also displayed the above mentioned antagonistic defense responses, phenotypically mimicking the triple receptor mutant. PSKα pretreatment of *tpst-1* leaves lead to a partial restoration of the resistance phenotypes, indicating that perception of PSKα has a direct effect on plant defense. These results suggest a mechanism whereby sulfated peptide perception by the PSKR LRR-RLK subfamily leads to an integration of growth-promoting and defense signals to modulate cellular plasticity for adjustment to environmental changes.

CS11-1

The wheat Mla homologue TmMla1 exhibits an evolutionary conserved function against powdery mildew in both wheat and barley

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The race-specific barley powdery mildew (*Blumeria graminis f. sp. hordei*) resistance gene *Mla* encodes CC-NB-LRR type resistance proteins. Genetic studies in breeding material have identified a large number of functional resistance genes at the *Mla* locus. Inter-generic allele mining resulted in the isolation and characterisation of an *Mla* homologue from diploid wheat, designated *TmMla1*, which shares 78% identity with barley HvMLA1 at the protein level. *TmMla1* was found to be a functional resistance gene against *Blumeria graminis f. sp. tritici* in wheat, hereby providing an example of *R* gene orthologs controlling the same disease in two different species. Interestingly, TmMLA1 was not functional in barley transient assays against a limited set of barley powdery mildew isolates. Replacement of the TmMLA1 LRR domain with that of HvMLA1 revealed that this fusion protein conferred moderate resistance against *B. graminis f. sp. hordei* isolate K1 in barley. Thus,TmMLA1 not only confers resistance in wheat but possibly also in barley against an as yet unknown barley powdery mildew race. The conservation of functional *R* gene orthologs over at least 12 million years is surprising given the observed rapid breakdown of *Mla*-based resistance against barley mildew in agricultural ecosystems. This suggests a high stability of *Mla* resistance in the natural environment before domestication. In analogy to *Mla*, homologues of the race-specific *R* gene *Pm3* (wheat) are isolated from barley and analysed for their response against wheat powdery mildew. The aim is to establish a wheat-barley-powdery mildew model system to investigate resistance responses against powdery mildew.

CS11-2

Allele pyramiding of the wheat powdery mildew resistance gene *Pm3*: A strategy for more durable resistance?

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Pyramiding genes of interest in elite crop cultivars is an important strategy to improve plant performance by breeding. In resistance breeding, this allows the combination of genes effective against different races or species of pathogens. Since single, race-specific resistance genes (*R* genes) are rapidly overcome, gene or allele stacks against a single pathogen are expected to render resistance more durable. Stable combination of alleles in a homozygous state is only possible in transgenic plants. In wheat, alleles of the *R* gene *Pm3* confer resistance against a broad variety of powdery mildew isolates (*Blumeria graminis* f.sp. *tritici*, *Bgt*).In our group, eight different *Pm3* alleles have been stably transformed in wheat and proven to be functional over multiple generations. We crossed these transgenic lines with each other to test whether *Pm3* alleles can be combined successfully. Infection tests with double homozygous plants and *Bgt* isolates that differentiate the resistance reaction of each parent showed that about half of the allele combinations exhibited additive resistance. However, in the remaining allele combinations a loss of resistance function of one of the two alleles was observed. This indicates that at least for some allele combinations suppression between *Pm3* alleles takes place, which reduces the effectiveness of *Pm3* pyramiding. Further experiments indicate that interference takes place at the protein level and that the LRR domain may be responsible for the suppression. Knowledge on the molecular basis of *R* gene suppression could be important to overcome the potential limitations by these effects for efficient resistance breeding.

CS11-3

The genome of the fungus *Cladosporium fulvum* suggests an ancestral host jump to tomato

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Cladosporium fulvum is a Dothideomycete fungus pathogenic on tomato but its biotrophic lifestyle differs from most other members of this class of fungi. Its genome sequence is most related to *Dothistroma septosporum*, a hemi-biotrophic pathogen causing pine needle blight and producing the toxin dothistromin. The *C. fulvum* genome size is twice that of *D. septosporum* because of invasion by transposable elements that have strongly shaped its structure and likely the interaction with its host plant tomato. Although it is a biotroph, the *C. fulvum* genome contains many genes that are typically found in hemi-biotrophs and necrotrophs. In particular, its carbohydrate-degrading enzyme catalog comprises a large arsenal for pectin degradation and *C. fulvum* grows well on different complex carbohydrate substrates including lignin. Also 15 gene clusters for secondary metabolite biosynthesis are present in the genome, including the gene cluster responsible for dothistromin production. Strikingly, several of the genes involved in cell wall-degradation and secondary metabolite production are not expressed in planta and others are pseudogenized. These phenomena are reminiscent of a jump by an ancestral *D. septosporum*-related fungal pathogen to tomato where it adapted to a biotrophic lifestyle by differentiation in gene content and gene regulation. Genes involved in adaptation to this lifestyle may encode not only small secreted effectors, but also structural proteins like hydrophobins and enzymes involved in degradation of antimicrobial saponins like α-tomatinase.

CS11-4

Protecting forest crops from disease: can comparative genomics provide management solutions?

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Commercial forest crops have lifespans measured in decades, but are equally susceptible to diseases as conventional short-lived food crops. An emerging problem is that the incidence of some foliar diseases is increasing due to climate change. For example, epidemics of Dothistroma needle blight (DNB) of pines in Europe and Canada are associated with increased rainfall. New methods of disease management are needed but difficulties inherent in working with this forest pathosystem have slowed progress. A breakthrough occurred recently when the genome of the DNB causal agent, *Dothistroma septosporum*, was sequenced by the Joint Genome Institute. Availability of the genome facilitated research that showed *D. septosporum* to be a hemi-biotroph rather than a necrotroph as previously supposed. Furthermore, the genome of *D. septosporum* is remarkably similar to that of the biotrophic tomato pathogen *Cladosporium fulvum*. Comparative analysis of genomes from these species revealed differences in genes for secondary metabolite biosynthesis and carbohydrate degradation that may help to determine host specificity. Genes common to the two

species are also of interest. *D. septosporum* has putative effector genes that appear to be functional orthologs of the well-studied *C. fulvum Avr* and *Ecp* genes. Although resistant races are not well defined in pine populations, some pine species have R genes that function in a gene-for-gene manner in a rust pathosystem. This raises the possibility that candidate *D. septosporum* effectors, along with other similar gene products identified from the genome, may be used to screen for DNB resistance in pine accessions.

CS11-5

Transgenic potato plants expressing WRKY8 transcription factor show resistance to potato blight pathogens

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We reported that *Nicotiana benthamiana* WRKY8 transcription factor is a physiological substrate of defense-related MAPKs, SIPK, NTF4 and WIPK. The phospho-mimicking mutant of NbWRKY8 induces *3-hydroxy-3-methylglutaryl CoA reductase 2* (*HMGR2*), which catalyzes the rate-limiting step in the biosynthesis of sesquiterpenoid phytoalexins. Here we investigated the role of *StWRKY8*, a potato ortholog, in the defense responses in the potato. The expression level of *StWRKY8* was transiently induced after inoculation with an avirulent isolate of potato late blight pathogen *Phytophthora infestans*. Ectopic expression of *StWRKY8* driven by a pathogen-inducible promoter in *N. benthamiana* showed resistance to a potent pathogen *Colletotrichum orbiculare*, indicating that StWRKY8 can confer resistance to pathogens. We generated transgenic potato plants expressing *StWRKY8* under the control of the pathogen-inducible promoter. The up-regulation of genes for *HMGR2* and *sesquiterpene cyclase*, which is a key branch enzyme of sesquiterpenoid phytoalexin synthesis, was observed in response to a virulent isolate of *P. infestans*. The virulent isolate of *P. infestans* and early blight pathogen *Alternaria solani* induced browning in mesophyll cells at the infection sites of the transgenic plants. Biomasses of both pathogens were reduced in the transgenic plants compare with wild-type plants. Thus, WRKY8 transgenic plants exhibited resistance to both near-obligate hemibiotrophic and necrotrophic pathogens.

CS11-6

Resistance genes within the same TIR-NBS-LRR locus from a wild North American grapevine species confer resistance to powdery mildew and downy mildew in cultivated grapevine

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The cultivated grapevine, *Vitis vinifera*, is highly susceptible to the fungal pathogen powdery mildew (*Erysiphe necator*) and the oomycete pathogen, downy mildew (*Plasmopara viticola*). Both of these biotrophic pathogens are economically important diseases of viticulture worldwide. *E. necator* and *P. viticola* were introduced into Europe from North America during the 1800s and as a result the Eurasian species *V. vinifera* has little genetic resistance to either pathogen. Due to the use of elite wine cultivars it can be undesireable to introduce resistance genes from wild grapevine relatives through classical breeding techniques. The *RUN1/RPV1* locus which originates from the wild North American grapevine species *Muscadinia rotundifolia* was previously found to confer resistance to both grapevine powdery and downy mildew following introgression into a *V. vinifera* background. Fine-scale genetic mapping localised *RUN1* and *RPV1* resistance to a region containing seven full-length TIR-NBS-LRR type resistance (R) gene candidates. These R-gene candidates have been transformed into susceptible *V. vinifera* cultivars including Shiraz, Portan and Tempranillo. This has allowed us to identify and functionally characterise the first powdery mildew (*RUN1*, Resistance to *Uncinula necator*, syn. *E. necator*) and downy mildew resistance (*RPV1*, Resistance to *Plasmopara viticola*) genes in grapevine. Cloning of *RUN1* and *RPV1* revealed that these R genes undergo alternative splicing across a cryptic intron to produce four transcripts, three of which are truncated. Both full length R gene products show nuclear localisation which is due to the presence of a C-terminal nuclear localisation signal (NLS).

CS12-1

Plant recognition of chitin and lipo-chitin signaling molecules

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It is now well established that chito-oligosaccharides (CO) and lipo-chitooligosaccharides (LCO) are potent signal molecules in plants. CO signals are recognized by plants leading to induction of basal resistance to invading fungal pathogens. In contrast, LCO signals, produced either by rhizobia or mycorrhiza, are recognized by plants and facilitate the establishment of symbiotic interactions. An interesting question is how plants can recognize very similar molecules but respond in such different ways. It is clear that LysM domain receptor-like kinases are involved in recognizing both CO and LCO signals and, hence, the two systems are likely evolutionarily linked. Our laboratory is focused on understanding the differences between CO and LCO signaling, characterizing the recognition steps, defining the downstream signaling processes and unraveling the extensive complexity that exists. Recent results suggest that for both CO and LCO signaling at least two receptor proteins are involve, only one of which has an active kinase domain. In the case of CO signaling, CERK1 is essential but appears to interact with other proteins depending on the specific biological context. Indeed, we have now identified or infer many layers of complexity in CO signaling, depending on the chemistry of the CO, which proteins comprise the receptor complex and how the pathogen modulates these processes.

CS12-2

Yin and yang of effector-triggered immunity: the negative regulator SRFR1 interacts with the positive regulator EDS1 and with resistance proteins

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Plant innate immune responses are tightly regulated because uncontrolled activity of the immune system leads to severe growth and developmental abnormalities. Sensing the presence of a specific pathogen effector by a cognate resistance protein is the trigger for one branch of the plant immune system, effector-triggered immunity (ETI). Whereas many effector-resistance protein systems have been identified, molecular processes that activate, signal and regulate ETI remain largely unknown. We had previously reported the identification and characterization of Arabidopsis SUPPRESSOR OF rps4-RLD (SRFR1), a negative regulator of AvrRps4- and HopA1-triggered immunity, two effectors that are usually recognized by the TIR-NB-LRR resistance proteins RPS4 and RPS6, respectively. In Col-0, absence of SRFR1 also leads to enhanced basal immunity, most prominently via activating the TIR-NB-LRR protein SNC1. Here, we identify cytoplasmic membrane-associated complexes of SRFR1 with RPS4, RPS6 and SNC1, and with ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1). A known positive regulator of basal immunity, EDS1 is also essential for ETI mediated by TIR-NB-LRR resistance proteins. Interestingly, AvrRps4 and HopA1 target EDS1 and disrupt its associations with SRFR1, RPS4 and RPS6. These effector-induced molecular perturbations likely form the basis for initiating ETI signaling. Our studies identify EDS1 as a direct virulence target of pathogen effectors that is guarded by some TIR-NB-LRR resistance proteins, and provides a molecular basis for negative regulation of ETI by SRFR1. Funded by NSF IOS-0715926 and IOS-1121114.

CS12-3

EDS1 connects pathogen effector recognition to cell compartment-specific immune responses

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Plants have evolved a sophisticated innate immune system to resist pathogen attack. Detection of pathogen effector proteins is mediated by intracellular nucleotide-binding leucine-rich-repeat (NB-LRR) receptors. The TIR-NB-LRR receptor class requires basal resistance regulator EDS1 to activate defense responses. We have investigated mechanisms linking receptor activation to downstream defense reprogramming. We show that Arabidopsis EDS1 connects recognition of *Pseudomonas syringae* type III effector AvrRps4 by TIR-NB-LRR receptor RPS4 to distinct defense outputs. RPS4 resides in a complex with EDS1 in tobacco nuclei after transient coexpression and in Arabidopsis leaf extracts after resistance activation, suggesting that EDS1 molecularly links RPS4 activation to downstream pathways. We also find that AvrRps4 interacts with EDS1 in tobacco nuclei indicating that EDS1 might be the virulence target of AvrRps4. We determined in which subcellular compartment AvrRps4 induces defense responses by forcing AvrRps4 localization to the host cytoplasm or nucleus. Strikingly, nuclear localization of AvrRps4 is sufficient to locally restrict bacterial growth whereas host cell death and transcriptional defense amplification leading to systemic resistance require nucleo-cytoplasmic AvrRps4. We propose that RPS4 engages EDS1 to intercept AvrRps4 and transduce receptor activation to qualitatively and spatially different immune outputs. We are now exploring whether EDS1 is the virulence target of AvrRps4 and whether AvrRps4 modifies EDS1 to compromise TIR-NB-LRR-mediated resistance. We are also determining whether EDS1 is guarded by RPS4 or recruited to the activated RPS4 receptor to trigger downstream defense.

CS12-4

***Arabidopsis Non-race specific Resistance-1 Disease (NDR1)* is required for robust activation of drought tolerance and PAMP triggered immunity via an abscisic acid dependent pathway**

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Much of the progress made towards the identification of a specific cellular role for Arabidopsis (*Arabidopsis thaliana*) NON-RACE-SPECIFIC DISEASE RESISTANCE1 (NDR1) has focused on effector-triggered immunity (ETI) signaling. Previous work in our laboratory provided the first mechanistic understanding of the global physiological role of NDR1 in plasma membrane-cell wall adhesion and its impact on disease resistance to *Pseudomonas syringae*. With distinct physiological and effector-dependent signaling roles for NDR1 established, our present study focuses on the link between NDR1 and stomata response, *via* an abscisic acid (ABA) dependent pathway that ultimately leads to a drought stress tolerance and PAMP triggered immunity (PTI). We analyzed the effects of drought stress on the relative water content (RWC %) of leaves over time, as well as the gene expression of key regulators in the ABA metabolic pathway. Furthermore, a role for NDR1 in the regulation of stomatal closure in response to external ABA was found. Seed germination was also affected by different concentrations of this hormone. Additionally, it was also observed

stomatal closure after treatment with the PAMP flg22. Furthermore, the loss of NDR1 alters the delivery of the *P. syringae* effector AvrRpt2 to the cell interior by the type-three secretion system. These findings, together with previous results, have allowed us to hypothesize that NDR1 fulfills a physiological and/or signaling role, not only in ETI, but also in the response to drought stress and pathogen entry. In total, our data suggest NDR1 mediates cross-talk between disease resistance and abiotic stress signaling.

CS12-5

Timing of innate immunity by the circadian clock in Arabidopsis

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The timing of effective defense responses against invading pathogens is crucial for plant fitness. The circadian clock integrates temporal information with environmental cues, such as light and temperature, in regulating plant growth and development. Recently, the circadian clock has been shown to affect plant responses to biotic cues. To further examine a role of the circadian clock in regulating plant immunity, we tested disease resistance in mutants disrupted in CIRCADIAN AND CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), two critical components of the central oscillator synergistically contributing to the circadian clock. We found that cca1 and lhy mutants synergistically affect basal and resistance gene-mediated defense against the bacterial pathogen *Pseudomonas syringae* and the oomycete pathogen *Hyaloperonospora arabidopsidis* (Hpa). Arrhythmicity of the circadian clock caused by overexpression of CCA1 or LHY resulted in severe disease susceptibility to *P. syringae*. We identified a downstream target of CCA1 and LHY, GLYCINE-RICH RNA-BINDING PROTEIN (GRP7), previously shown to influence plant defense and stomatal activity and as a key constituent of a slave oscillator regulated by the circadian clock. We show that the defense role of CCA1, LHY, and GRP7 against *P. syringae* is at least partially through circadian control of stomatal aperture. Furthermore, we found defense activation by *P. syringae* infection and treatment with flg22 (an elicitor of basal defense) can also feedback-regulate clock activity. Together these data strongly support a role of the circadian clock in defense control and reveal reciprocal crosstalk between the circadian clock and plant innate immunity.

CS12-6

Repeated evolution of genetic incompatibilities involving a single NB-LRR gene cluster: lessons from hybrid necrosis studies in *Arabidopsis thaliana*

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Hybrid necrosis in plants, a commonly observed phenomenon in crosses of divergent lineages, is characterized by autoimmune responses that can potentially generate gene flow barriers. We exploit F_1 hybrid necrosis (incompatibility) in *Arabidopsis thaliana* as a tool to study fitness effects of immune system diversity in plant population. Our studies identified a single cluster of NB-LRR encoding genes, *DANGEROUS MIX2 (DM2)/RPP1*, as the cause for multiple, independently evolved genetic incompatibilities in *A. thaliana*. The *DM2d* gene in the Uk-1 strain, which likely arose through within-cluster duplication events, interacts with the Uk-3 allele of the unlinked NB-LRR locus *DM1/SSI4*. The Bla-1 allele of *DM2h*, which is found in a rare syntenic position in the cluster (with clear orthologs in many accessions), interacts with the Hh-0 allele of *DM3*, which encodes a peptidase. The causal changes in the *DM1* and *DM2h* genes are in the C-termini, which include the highly variable LRR domains. Profiling of the *DM1* and *DM2* clusters using short reads from numerous accessions provided a nuanced picture of the structural complexity of these genomic regions. Although specific incompatibility alleles are rare, there is extended haplotype sharing between accessions with the necrosis-inducing alleles, suggestive of selective pressures that maintain these alleles. Our results suggest that both functional and structural features interact to make specific genomic regions, such as the *DM2* cluster, particularly likely to generate genetic incompatibilities. In addition, our studies reveal new mechanistic details of how immune receptors are activated by identifying new NB-LRR interactors.

CS13-1

Molecular aspects of defense priming

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Plants can be primed for more rapid and robust activation of defense to biotic or abiotic stress. Priming follows perception of molecular patterns of microbes or plants, recognition of pathogen-derived effectors or colonization by beneficial microbes. However the process can also be induced by treatment with some natural or synthetic compounds and wounding. The primed mobilization of defense is often associated with development of immunity and stress tolerance. Although the phenomenon has been known for decades, the molecular basis of priming is poorly understood. I will summarize recent progress made in unraveling molecular aspects of defense priming that is the accumulation of dormant mitogen-activated protein kinases and chromatin modifications in the promoters of defense genes. I will also discuss the potential of plant defense priming for application in the field. References: Beckers & Conrath (2007) Curr Opin Plant Biol 10: 425-431; Beckers et al. (2009) Plant Cell 21: 944-953; Jaskiewicz et al. (2011) EMBO rep 12: 50-55; Conrath (2011) Trends Plant Sci 16: 524-531.

CS13-2

Effector modulation of the Arabidopsis actin cytoskeleton by *Pseudomonas syringae*

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The plant actin cytoskeleton plays a critical role in a variety of cellular processes, including development, cell organization and innate immune signaling. Recent work in our laboratory has identified a role for the actin cytoskeleton in defense of the phytopathogenic bacterium *Pseudomonas syringae*. By first screening a panel of actin binding protein (ABP) mutant lines from Arabidopsis, we mapped the signaling network(s) required for resistance mediated through polymerization and depolymerization of G- and F-actin filaments, respectively. This work has identified a direct link between pathogen perception, actin depolymerization and the regulation of transcription of a number of NB-LRR resistance genes. In total, we have identified a requirement of nuclear-actin dynamics in the control of R-gene expression and function. To further this work, and to elucidate the specific signaling of resistance and virulence through modulation of the host actin cytoskeleton, we have utilized a high-throughput confocal microscopy-based screen for the identification of effector targeting of the Arabidopsis actin cytoskeleton. Using a combination of cell biology, genetic and biochemical analyses, we have screened a panel of ABP mutants for targeting of the cytoskeleton by *P. syringae*. In brief, we have identified *P. syringae* DC3000 effector proteins that specifically modify the host actin cytoskeleton 24 hours after infection. Our data suggestion a strong correlation between host actin modification in plant-pathogen interactions and that previously characterized in mammalian-pathogen interactions.

CS13-3

Dynamics and biological significance of RNA-directed DNA methylation in plant immunity

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In higher eukaryotes, the vast majority of the genome appears to be transcribed, leading to an extraordinary diversity of non-coding RNAs (ncRNAs). Whereas the functional significance of these ncRNAs is mostly unknown, increasing evidence suggests a role for these molecules in guiding chromatin modifications. In plants, a large portion of ncRNAs is processed by the RNA silencing machinery to produce siRNAs that guide cytosine DNA methylation of repeated sequences such as transposable elements leading to their transcriptional silencing. This phenomenon is referred to as RNA-directed DNA methylation (RdDM) and contributes to the transcriptional repression of some developmentally- as well as abiotic stress- regulated genes that carry repeats in their vicinity. Whereas our knowledge on the mechanisms of RdDM has rapidly increased over the past few years, little is known on the dynamics as well as biological roles of this pathway in physiological and ecological relevant processes such as plant disease resistance. Here, we provide evidence that RdDM negatively regulates the Arabidopsis innate immune response. Accordingly, we have identified immune-response genes that are controlled by RdDM and that carry repeats, and associated siRNAs, in their vicinity. I will present the dynamics of siRNA-directed epigenetic changes at those loci and report the biological significance of such regulatory process in the context of antibacterial defense. I will also present the extent to which bacterial effectors have evolved to interfere with this epigenetic pathway to enable disease.

CS13-4

Lack of susceptibility factors: a novel breeding strategy for non-host like resistance?

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Plant are continuously attacked by a broad diversity of pathogens. Breeding for resistance to pathogens has been focused on introducing resistance genes that encode proteins to recognize specific pathogen effector proteins leading to host resistance. This type of host resistance is frequently broken as new pathogen races constantly appear. Another type of resistance is non-host resistance, which describes the immunity of an entire plant species against all genetic variants of a pathogen species. Non-host resistance is yet unexploited in plant breeding. In order to overcome non-host resistance pathogens have to suppress plant innate immunity, for which pathogen effectors and their targeted host-factors play a central role. The absence of certain host-factors encoded by plant susceptibility genes (S-genes) enable plants to escape the defence-suppression and thus to maintain their non-host status. In Arabidopsis, genetic dissection of disease susceptibility to powdery and downy mildews has identified multiple S-genes whose impairment results in disease resistance in absence of severe fitness costs. Although several of these S-genes have been cloned and characterized in more detail it is unknown to which degree their function in disease susceptibility is conserved among different plant species. Here we show that Arabidopsis PMR4 and DMR1 encoding a callose synthase and homoserine kinase respectively have functional orthologs in tomato. Silencing of both genes using RNAi resulted in resistance to tomato powdery mildew, *Oidium neolycopersici*. Severe fitness costs were found associated with SlDMR1 but not with SlPMR4 silencing, indicating the latter has potential in disease resistance tomato breeding.

CS13-5

Molecular mechanisms for generation of NO and ROS in plant immunity

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Rapid production of reactive oxygen species (ROS) and nitric oxide (NO) has been implicated in plant immunity. A potato calcium-dependent protein kinase 5 (StCDPK5) activates NADPH oxidases StRBOHA to D by direct phosphorylation of their N-terminal regions, and heterologous expression of StCDPK5 and StRBOHs in *Nicotiana benthamiana* results in ROS burst. The transgenic potato plants that carry a constitutively active StCDPK5 driven by a pathogen-inducible promoter of the potato showed high resistance to late blight pathogen *Phytophthora infestans* accompanied by HR-like cell death and H_2O_2 accumulation in the attacked cells. In contrast, these plants showed high susceptibility to early blight necrotrophic pathogen *Alternaria solani*, suggesting that ROS burst confers high resistance to near-obligate hemibiotrophic pathogen *P. infestans*, but high susceptibility to necrotrophic pathogen. There are many reports about complementary, synergistic and overlapping functions of NO and ROS in the defense responses. Two mitogen-activated protein kinase (MAPK) cascades, MEK2-SIPK and cytokinesis-related MEK1-NTF6, are involved in the induction of *NbRBOHB* at the transcriptional level in *N. benthamiana*. On the other hand, NO burst is regulated by the MEK2-SIPK cascade. Conditional activation of SIPK in potato plants induces ROS and NO bursts, and confers resistance to both near-obligate hemibiotrophic and necrotrophic pathogens, indicating the plants may have obtained during evolution the signaling pathway which regulates both NO and ROS production to adapt to wide-spectrum pathogens.

CS13-6

Plant immu*NO*logy: Cracking the redox code

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Changes in redox status are a conspicuous feature of immune responses in a variety of eukaryotes, but the associated signalling mechanisms are not well understood. In plants, attempted microbial infection triggers the rapid synthesis of nitric oxide (NO) and a parallel accumulation of reactive oxygen intermediates (ROIs), the latter of which is generated by NADPH oxidases related to those responsible for the pathogen-activated respiratory burst in phagocytes. Both NO and ROIs have been implicated in immune signalling and the control of the hypersensitive response (HR), a programmed execution of plant cells at sites of attempted infection. Our findings suggest that S-nitrosylation, the addition of an NO moiety to a protein cysteine thiol to form an S-nitrosothiol, is a key regulator of the plant defence response, controlling ROI synthesis, the accumulation of the immune activator, salicylic acid (SA) and cognate SA signalling. We are employing a variety of complementary approaches, including: forward and reverse genetics, Solexa-based gene expression profiling and novel proteomics strategies, to uncover the molecular landscape of S-nitrosylation during plant immune function.

CS14-1

Harnessing TAL effector-DNA targeting to understand and prevent plant diseases caused by *Xanthomonas*

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Transcription activator-like (TAL) effectors are type III-secreted, DNA binding proteins used by *Xanthomonas* to activate plant genes that promote infection. Some TAL effectors activate genes that confer resistance associated with host cell death. The proteins contain polymorphic repeats that assemble into a superhelix to track the DNA major groove and make base specific contacts. A TAL effector-DNA binding code that links individual repeat types to individual bases, with some degeneracy, enables prediction or synthesis of TAL effector binding sites and customization of TAL effectors for binding new DNA sequences, accelerating discovery and enabling applications from targeted regulation to genome editing. Using the code and transcript profiling data, we identified multiple candidate targets in rice for TAL effectors of *X. oryzae* pv. *oryzicola*, which causes bacterial leaf streak of rice, and *X. oryzae* pv. *oryzae*, which causes rice bacterial blight, and experimentally validated roughly half that we tested further. Using TAL effector-based technologies, we discovered among these the first known gene for bacterial leaf streak susceptibility. Also, comparing validated and non-validated candidates yielded characteristics useful for better prediction and design. Using this information, we engineered a bacterial blight resistance gene to be activated by multiple oryzae and oryzicola TAL effectors. As a stable transgene, this construct provided resistance against diverse strains of both pathovars. We observed however, that the TAL effector binding sites contain sequences apparently under selection in rice promoters, suggesting endogenous regulatory roles that might activate cell death under some conditions, indicating a need for caution with this approach.

CS14-2

Phytoplasma effectors modulate plant development and plant-insect interactions

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Arabidopsis thaliana plants infected with the bacterial pathogen Aster Yellows phytoplasma strain Witches' Broom (AY-WB) exhibit witches' broom and leafy flower symptoms and promote reproduction rates of the AY-WB insect vector (the aster leafhopper *Macrosteles quadrilineatus*) by 60% compared to non-infected Arabidopsis plants. We previously sequenced the genome of AY-WB and identified 56 secreted AY-WB proteins (SAPs) that are candidate effector proteins. To investigate which effectors modulate plant development and leafhopper fitness, we generated stable transgenic Arabidopsis lines for these effectors. SAP11 Arabidopsis plants show crinkled leaves and increase in stem numbers resembling the witches' broom phenotype, while SAP54 plants exhibit leafy flowers and SAP05 plants long slender leaves and early flowering. We found that SAP11 binds and destabilizes Arabidopsis *CINCINNATA (CIN)*-related TCPs that are conserved plant transcription factors involved in plant development and positively regulate lipoxygenase (*LOX*) genes required for jasmonate (JA) synthesis. *LOX2* expression and JA production are downregulated in the SAP11 plants, and *M. quadrilineatus* produces significantly more progeny on these plants and on *LOX2*-silenced and *jar1* mutant Arabidopsis. Thus, SAP11 suppresses the plant defence response to the AY-WB leafhopper vector by destabilizing TCPs leading to an increase in insect vector progeny. As in nature AY-WB depends on these insects for transmission to other plants, we propose that *SAP11* is a vivid example of a gene that has an extended phenotype beyond the organism in which it resides, a concept put forward in Richard Dawkins' classic book "The extended phenotype - The long reach of the gene".

CS14-3

The trimeric autotransporter adhesin XadA is localized in outer membrane vesicles and mediates attachment to surfaces and suppress cell-cell aggregation in *Xylella fastidiosa*

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While virulence of *Xylella fastidiosa* to grapevines requires migration and spread within the xylem, its transmission by xylem sap-feeding insects is fostered by expression of attachment factors. XadA is a trimeric autotransporter adhesin that is secreted into the extracellular milieu and whose expression is high *in-planta*. Using deconvolution microscopy to assess immunolocalization we find that XadA is localized to both the outer membrane (OM) and outer membrane vesicles (OMVs). While XadA expression is enhanced by DSF-mediated quorum sensing and cyclic di-GMP signaling its secretion is suppressed in the presence of DSF. Deletion of *xadA* impaired insect transmission but did not affect bacterial virulence to grapevine. While heterologous expression of *xadA* in *Escherichia coli* increased the attachment of cells to surfaces, it did not increase cell-cell aggregation. A *X. fastidiosa xadA* deletion mutant was more adhesive to surfaces and more self-aggregative than the wild type strain apparently due to compensatory increases in abundance of other adhesins in the mutant. Thus, although it attaches to surfaces, XadA function also prevents self-aggregation. Since it can be localized to both the OM and OMVs we hypothesize that under conditions of high DSF levels XadA is retained, enhancing the adhesiveness of cells, thereby facilitating transmission by insects, while at low DSF levels XadA-containing OMVs are released where they serve as virulence factors coating xylem vessels walls and preventing attachment and aggregation, thereby promoting migration and colonization of the host plant.

CS14-4

Towards a life history model of *Pseudomonas syringae* pv. *tomato* that integrates adaptations to habitats beyond the plant apoplast

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The most intensively studied part of the life history of *Pseudomonas syringae* pv. *tomato* (Pto) is its behavior inside the tomato leaf apoplast while causing bacterial speck. Little is known about the life of Pto before reaching the apoplast and its life after having caused disease. Sampling of *P. syringae* from precipitation, snow pack, leaf litter, and surface water in non-agricultural areas of France and New Zealand has revealed the existence of *P. syringae* strains that are as aggressive on tomato as Pto strains isolated from diseased tomatoes. These strains are genetically very similar to Pto; in particular, strains indistinguishable from strain DC3000 have been found in a creek in New Zealand. We report genetic, genomic, and phenotypic comparison of these strains with typical Pto strains to infer the evolutionary history of Pto and the possible connection of Pto life history with the water cycle. Additionally,

genomic comparison among Pto strains revealed mutational hot spots in genes coding for methyl-accepting chemotaxis proteins suggesting an important role of chemotaxis in the life history of Pto. We show experimental evidence of the importance of chemotaxis for leaf invasion and for life in the apoplast. We finally report on preliminary results on the life of Pto after its departure from diseased plants and lay out how to develop and test an integrative model of the life history of Pto.

CS14-5

Systematic dissection of the *Agrobacterium* type VI secretion system reveals machinery and secreted components for subcomplex formation

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The type VI secretion system (T6SS) is widely distributed in pathogenic Proteobacteria. The evolutionary and structural analysis of T6SS reveals its resemblance of the T4 bacteriophage tail, in which an outer sheath structure contracts an internal tube for injecting nucleic acid into bacterial cells. However, how this phage tail-like T6SS structure is assembled in vivo and executed for exoprotein or effector secretion remains largely unknown. Here, we used a systematic approach to identify T6SS machinery and secreted components and investigate the interaction relationship among the putative sheath and tube components of *Agrobacterium tumefaciens*. We showed that a total of 14 T6SS components play essential roles for the secretion of the T6SS hallmark exoprotein Hcp. In addition, we discovered a novel *Agrobacterium*-specific T6SS exoprotein Atu4347 that is dispensable for Hcp secretion. Interestingly, the putative tube components Hcp and VgrG as well as the Atu4347 exoprotein are localized on bacterial surface. Atu4342 (TssB) and Atu4341 (TssC41) interact and stabilize each other, suggesting they are functional orthologs of TssB (VipA) and TssC (VipB), the sheath components identified in *Vibrio cholerae*. Importantly, TssB interacts directly with the three exoproteins, in which Hcp also interacts directly with VgrG-1 by co-purification from *Escherichia coli*. Further co-immunoprecipitation and pulldown assays revealed these subcomplex(es) in *A. tumefaciens* and thereby in support of T6SS functioning as a contractile phage tail-like structure.

CS14-6

Isolation of *Burkholderia glumae* resistance genes from rice using whole genome association mapping

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Burkholderia glumae, formerly known as *Pseudomonas glumae*, was first described in Japan as a causal agent of grain rot in rice. In last few years, this pathogen has been reported from major rice growing regions of the world. A large-scale study has been initiated to isolate the gene governing resistance to *Burkholderia glumae* in rice by utilizing naturally available genetic variation of diverse rice cultivars. This ongoing research will enable us to understand in depth the genetic/molecular mechanism involved in *B. glumae* disease resistance and will help to minimize this disease. Rice NAM population has been generated at Iwate Biotechnology Research Center using the cultivar Hitomebore as the common parent crossed with 22 world Oryza accessions representing wild genetic diversity. Screening of NAM population has been initiated after successful selection of the parent cultivars showing resistance or susceptibility/hyper susceptibility for *B. glumae* infection. Segregation test are being performed under green house conditions. Quantitative trait loci (QTL) analysis and whole genome association mapping will be conducted employing high-throughput genotyping of validated SNPs in the selected mapping population and phenotype so observed. Besides using NAM population, EMS (Ethyl Methane sulfonate) generated mutant lines of an elite rice cultivar Hitomebore will also be screened to identify novel rice genes involved in grain rot/seedling blight disease resistance.

CS15-1

Regulation of innate immunity in barley-powdery mildew interactions

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Genes encoding early signaling events in pathogen defense often are identified only by their phenotype. Such genes involved in barley-powdery mildew interactions include *Mla*, specifying race-specific resistance; *Rar1 (Required for Mla12-specified resistance1)*, and *Rom1 (Restoration of Mla-specified resistance1)*. The HSP90-SGT1-RAR1 complex appears to function as chaperone in MLA-specified resistance, however, much remains to be discovered regarding global signaling underlying plant immunity. Genetic analyses of fast-neutron mutants derived from CI 16151 (*Mla6*) uncovered a novel locus, designated *Rar3 (Required for Mla6-specified resistance3)*. *Rar3* segregates independent of *Mla6* and *Rar1*, and *rar3* mutants are susceptible to *Blumeria graminis* f. sp. *hordei (Bgh)* isolate 5874 (AVR_{a6}), whereas, wild-type progenitor plants are resistant. Seven-day old seedlings (PO:0007094) from the *rar3* mutant and wild-type progenitor were inoculated with *Bgh* 5874, harvested at 16 and 32 HAI, and subjected to both Barley1 GeneChip and RNA-Seq analyses. A randomized block design with two independent biological replications was used to obtain expression measurements. The resulting data sets are being used for two purposes; 1) transcript-based isolation of the gene(s) responsible for the *rar3* phenotype, and 2) an assessment of *rar3*-mediated transcriptome reprogramming in both compatible and incompatible interactions in response to challenge with the biotrophic pathogen, *Bgh* 5874. Whereas *Rar1* affects transcription of only a few genes; *Rar3* appears to influence thousands, notably in genes controlling ATP binding, catalytic activity, transcription, and phosphorylation; possibly membrane bound or in the nucleus. Research supported in part by NSF Plant Genome grant 0922746.

CS15-2

Properties and structure of the plant immune signaling network

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The plant immune signaling network is different from other plant signaling networks because pathogens not only initiate signaling events but have also been rapidly evolving to interfere with plant signaling. Therefore, the plant immune signaling network must have properties that allow it to withstand perturbations from a wide variety of pathogens without heavily relying on evolutionary adaptation. Unnecessary immune responses carry negative impacts on plant fitness, further constraining possible network properties. Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are two well-defined modes of plant immunity. PTI is initiated by recognizing molecular patterns common among related microbes, including pathogens and benign microbes. Pathogens well-adapted to a host plant deliver effectors into the plant cell that interfere with PTI signaling and negate PTI. Plants may have receptors that recognize some of the pathogen effectors and trigger ETI, resulting in immunity. We demonstrated that at least some cases of PTI and ETI extensively share the signaling machinery and that what distinguishes PTI and ETI is the way the common signaling network operates. There is synergy among signaling sectors in PTI and compensation in ETI. The latter explains the robustness of ETI. In ETI compensation does not result from simple redundancy among sectors but likely mediated by prevalent negative regulatory relationships between different signaling sectors. We are currently modeling signaling dynamics among immune signaling sectors to understand how the same signaling network machinery can result in different network properties observed in PTI and ETI.

CS15-3

Rapid Nod factor-induced changes in the phosphoproteome and the transcriptome of *Medicago truncatula*

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Establishment of symbiotic associations between legumes and nitrogen-fixing rhizobia commences with the perception, by the host plant, of bacterial lipochitooligosaccharides known as Nod factors (NF). Recognition of NF by plasma membrane receptor kinases triggers rapid cellular and molecular responses that culminate within one hour, in the activation of a nuclear calcium/calmodulin-dependent protein kinase called DMI3, and the regulation of gene expression. Despite great progress in genetic analyses, there has been little large-scale biochemical characterization of the early molecular events in this signaling cascade. We report here extensive tandem mass spectrometric-based, untargeted measurements of rapid NF-induced changes in the phosphorylation status of 13,506 phosphosites, in 7,739 proteins from *Medicago truncatula*. In order to place these changes within a biological context, untargeted quantitative phosphoproteomic and RNA measurements in wild-type plants were compared with those observed in two mutants, one defective in NF perception (*nfp*) and one defective in nuclear signal transduction events (*dmi3*). These experiments have revealed the identity of phosphosites within several hundred phosphorylated proteins that appear to be specifically associated with NF-signaling. In addition, these experiments have revealed an additional layer of complexity involving *dmi3*-mediated feedback mechanism, and cryptic NF-receptors, probably involving those required for mycorrhizal signal perception.

CS15-4

Ceramide accumulation plays a key role in Arabidopsis programmed cell death

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Arabidopsis plants that lack ceramide kinase (ACD5) eventually display spontaneous programmed cell death (PCD) and are more susceptible to certain pathogens. Here, we report our study of ceramide accumulation kinetics, ultrastructural changes and gene expression in *acd5* plants during pathogen infection. Using quantitative sphingolipid profiling, a high level of ceramides was

found concomitant with the appearance of the spontaneous cell death phenotype in *acd5* mutants, suggesting that accumulation of ceramides is important for PCD. Moreover, expression of defense-related PR genes, ROS related genes, senescence marker genes, and autophagy-related genes were also detected late in the development of *acd5*. When younger plants were infected with *Botrytis cinerea* (before spontaneous PCD), much higher levels of ceramide accumulated in acd5 plants when compared with wild-type plants. Our results indicate that ceramide accumulation is highly correlated with the spontaneous cell death phenotype and increased susceptibility to *Botrytis cinerea* in *acd5* plants. The possibility role of ceramide in plant PCD will be discussed.

CS15-5

Two novel transcription factors regulating MAMP-elicited phenylpropanoid metabolism in Arabidopsis

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It has been known for some time that recognition of bacterial MAMPs such as flagellin by plants elicits the production of phenylalanine-derived secondary metabolites, known collectively as phenylpropanoids. This pathway, including the production of the phenolic polymer lignin, is highly conserved throughout plant evolution, and its regulation is a primary concern for biofuel crops. However, the transcriptional networks that coordinate the biosynthetic genes remain largely uncharacterized. From available microarray data in the model plant *Arabidopsis thaliana*, two transcription factors were identified to be highly upregulated by the bacterial MAMP flagellin, one of the MYB class and one of the ERF class. Here we propose a role for these transcription factors in regulation of defense-elicited phenylpropanoid metabolism. We demonstrate this through qPCR experiments on inducible overexpression lines, complemented with metabolic profiling via HPLC-MS. The MYB-type TF appears to regulate MAMP-inducible lignin biosynthesis, regulating transcription of the biosynthetic genes and exhibiting reduced MAMP-elicited lignin when silenced. The ERF-type TF regulates anthocyanin production and when silenced, plants are rendered susceptible to non-host pathogens. Together, these two proteins control defense-related phenylpropanoid metabolism in a complementary manner. The results of these experiments should provide new insight to the regulation of the phenylpropanoid pathway in defense.

CS15-6

Signaling in soybean defense responses

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Improved protection of crop plants against pathogens will require detailed understanding of the genes mediating pathogen recognition and defense responses in each species. In soybean, recent advances in genomic resources and the availability of functional genomics tools have enabled systematic characterization of defense signaling pathways that build upon frameworks established in model plants such as Arabidopsis. We have used gene expression profiles, knowledge from model systems, and sequence information of known resistance loci to generate a library of virus-induced gene silencing (VIGS) constructs in a DNA-based *Bean pod mottle virus* vector. The VIGS constructs have been tested for the roles of their target genes in regulating soybean defenses. In a screen targeting soybean MAP kinase (MAPK) cascades, silencing of soybean MAP kinase 4 (*GmMPK4*) strongly activated constitutive defense responses including cell death, increased salicylic acid levels, increased expression of defense-related genes, and decreased expression of genes that promote plant growth. *GmMPK4*-silenced plants were more resistant to *Soybean mosaic virus* (SMV) and downy mildew than vector control plants confirming its function as a negative regulator of defenses to biotrophic pathogens. In an independent screen, eight genes were identified as required for *Rsv1*-mediated resistance to SMV. These genes include *Rsv1* candidate genes, *GmEDR1*, *GmEDS1*, *GmHSP90*, *GmJAR1*, *GmPAD4* and two WRKY transcription factors. This direct reverse functional genomics approach has enabled us to gain insight into signaling modules regulating soybean defenses against diverse pathogens.

CS16-1

Involvement of a novel class of NB-LRR proteins in disease resistance

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Plant genomes encode large numbers of nucleotide-binding, leucine-rich repeat (NB-LRR) proteins which make up a one branch of the plant innate immune system through recognition of pathogen-encoded effector proteins. NB-LRR proteins fall into two broad classes: those with a Toll and interleukin-1 receptor (TIR) domain at their N-terminus and those with a coiled-coil (CC) domain at the N-terminus. Although a number of recent studies have yielded insights into how NB-LRR recognize their cognate effectors, the molecular events that take place post-recognition signaling is less clear. We have identified a basal clade of NB-LRR proteins that is distinguished from all others by having CC domains resembling the *Arabidopsis thaliana* RPW8 protein, which we refer to as CCR domains. We find that CCR-NB-LRR-encoding genes are present in the genomes of all higher plants surveyed, and that they comprise two distinct subgroups: one typified by the *Nicotiana benthamiana* N-required gene 1 (NRG1) protein and the other by the Arabidopsis activated disease resistance gene 1 (ADR1) protein. Consistent with previous reports, our results suggest that these proteins are required downstream of canonical NB-LRR proteins and are thus likely to play a role in signaling rather than in effector recognition. This is further supported by the finding that, in contrast to CC-NB-LRR proteins, the CCR domains of both NRG1- and ADR1-like proteins are sufficient for the induction of defense responses, including anti-viral responses in the absence of cell death.

CS16-2

Proteomic and genetic analyses of plant immune complexes

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The plant innate immune system is capable of recognizing diverse microbial patterns and pathogen effectors through intracellular and surface-localized immune receptors. The plant protein RIN4 plays a key role in immune signaling in Arabidopsis, tomato, lettuce, and soybean. The *Arabidopsis* RIN4 protein is targeted by multiple pathogen effectors and is guarded by the plant NLR immune receptors RPM1 and RPS2. Furthermore, RIN4 also plays a role in PAMP defense responses and pathogen entry through stomatal apertures. We hypothesize that RIN4 acts as an adapter protein, bridging interactions between important immune signaling proteins. Proteomic analyses of RIN4 protein complex constituents reveals dynamic changes in RIN4 protein complexes in response to stimulus with the bacterial pathogen *Pseudomonas syringae*. The role of RIN4 phosphorylation in triggering activation of the *Arabidopsis* immune receptor RPM1 will be presented. The importance of RIN4 phosphorylation during compatible interactions will also be presented. Collectively, results indicate that posttranslational modification of RIN4 induces dynamic changes in its interactions with key immune signaling proteins.

CS16-3

Danger sensing and signaling via an endogenous elicitor/receptor system in *Arabidopsis*

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Recognition of microbe-associated molecular patterns (MAMPs) via cell-surface receptors such as FLS2 and EFR is central to initiate MAMP-triggered immunity (MTI) that restricts multiplication of potentially infectious microbes. However, how hosts distinguish pathogens from non-pathogens that share MAMPs remains largely unknown. We hypothesize that coincidental detection of MAMPs and danger signals, e.g. disruption of host cell integrity and/or perturbation of MAMP-triggered signaling, acts as a potent trigger for immune response against pathogens. Our genetic work in Arabidopsis points to a critical role of sustained, rather than initial, transcriptional reprogramming for effective MTI activation. The endogenous elicitor/receptor Pep/PEPR pathway has emerged as a target but also component of sustained MTI signaling. It has been described that recognition of the elicitor-active ligands Peps occurs through the cell-surface receptors PEPR1 and PEPR2, despite the lack of an N-terminal signal peptide for targeting the ligand precursors to the secretory pathway. This implies that Pep ligands released upon cellular damages activate PEPR signaling, but compelling evidence is missing for this model and for the significance of this system in host immunity. We provide genetic, biochemical, and genomics evidence that the Pep/PEPR pathway is tolerant to or rather enhanced under the conditions in which MAMP-triggered signaling is hampered. Together with our findings for a role of PEPRs in basal and systemic immunity and in the co-activation of otherwise antagonizing salicylate- and jasmonate-mediated immunity, we propose that the Pep/PEPR pathway serves as a fail-secure system in MTI and facilitates the engagement of different immune branches.

CS16-4

Lectin-mediated resistance as a novel and universal innate immunity toward plant viruses

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Plants possess a multilayered defense response known as plant innate immunity to infection by a wide variety of pathogens. Similar to the plant innate immunity against bacteria, fungi, and oomycetes, the defense machinery to plant viruses can be divided into multiple stages. The most extensively studied plant innate immune responses to viruses are the R protein-mediated resistance and RNA silencing. Lectins are sugar-binding proteins, so they have been regarded as self-nonself-discriminating molecules. Several kinds of lectins from animals as well as plants actually play essential roles in the innate immunity of animal cells. However, despite the fact that lectins were first identified in plants and that plants have evolved the largest families of lectins with heterogenous structures and activities, the detailed physiological roles of lectins in plant cells are unclear. Here we identified a novel lectin gene that confers resistance to potexviruses, members of the genus *Potexvirus*, using map-based positional cloning analyses. Since the lectin conferred resistance to potexviruses, we designated it *JACALIN-TYPE LECTIN REQUIRED FOR POTEXVIRUS RESISTANCE 1* (*JAX1*). JAX1-mediated resistance was independent of R protein-mediated resistance, RNA silencing and defensive plant hormone signaling pathways. Through the molecular characterization of JAX1, we revealed that lectins show a variety in the levels as well as their target viruses of resistance. Along with the distinct properties of the resistance from known resistance machineries, we suggest the generality of a definite class of plant innate immunity, lectin-mediated resistance (LMR).

CS16-5

Interplays between positive and negative transcriptional regulators mould NB-LRR protein triggered immunity

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Activation of plant immune receptors usually leads to defense reprogramming that involves the coordination of cellular transcriptional machinery. The barley MLA gene is polymorphic in nature and encodes NLRs of the coiled-coil (CC)-NB-LRR type that each detects a cognate isolate-specific effector of the barley powdery mildew fungus. We showed that MLA protein function in the nucleus to confer resistance against the powdery mildew fungus, and recently we reported that MLA induces cell death signaling in the cytoplasm, together our data suggest a bifurcation of MLA-triggered cell death and disease resistance signaling in a compartment-dependent manner. We recently identified a barley R2R3-type MYB transcription factor (named HvMYB1) with two DNA binding domain interacting with the CC domain of several MLA proteins in yeast, *in vitro* and *in vivo*. Significantly, these interactions appear to be dependent on MLA CC homodimerization. Moreover, HvMYB1 mutations diminishing MLA CC interaction also compromise its DNA binding activity. Interestingly, HvMYB1 also interacts specifically with barley WRKY1 but not WRKY2. These two WRKYs were previously found to interact with MLA CC domain and act as negative regulators in basal defense as well as MLA triggered defense responses. HvMYB1 overexpression in barley markedly enhanced MLA-mediated disease resistance, whereas knock-down of HvMYB1, achieved via BSMV-mediated VIGS or transiently induced gene silencing, compromised barley disease resistance against the fungus pathogen. Our study suggests that this type of NB-LRR proteins activates immune responses through the interplays of tightly controlled cellular transcriptional networks.

CS16-6

mRNA decay in kinase-mediated responses to pathogens

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Plants have evolved multi-layered defence responses, activated upon recognition of invading pathogens. One layer includes trans-membrane receptors that recognize evolutionarily conserved microbe-associated molecular patterns (MAMPs). Signalling via MAP kinases from these receptors leads to reprogramming of gene expression and production of host proteins for thwarting pathogenic intruders. MAMP-activated MAP kinase 4 (MPK4) regulates the expression of a subset of defence genes via a WRKY transcription factor. However, how plant MAP kinases regulate defence genes is still poorly understood. Recently we found an *in vivo* association in Arabidopsis between MPK4 and PAT1, a component of the mRNA decapping machinery. Interestingly, *pat1* mutants exhibit similar phenotypic characteristics to *mpk4* mutants, namely dwarfism and increased resistance toward bacterial pathogens. These data strongly suggest that MPK4 and PAT1 function together to regulate defense responses. mRNA decapping represents a critical step in eukaryotic mRNA turnover, and MPK4 is a regulatory node controlling transcriptional reprogramming via transcription factors. Thus, linking MPK4 to mRNA decay offers another efficient mechanism for this MAP kinase to regulate the rapid changes required to instigate defense responses. I am using genetics and biochemistry to probe the function of PAT1 and mRNA decapping in plant innate immunity, research that remains largely unexplored.

CS17-1

Transcriptional regulation for nodulation in legumes

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Formation of root nodules is one of the critical steps in successful interaction of nitrogen fixing soil bacteria with legumes. Upon recognition of the bacteria by host plants, a set of symbiosis genes is activated for intracellular signal transduction. Among them, CCaMK plays pivotal roles for nodulation. A gain-of-function mutant of CCaMK triggers nodulation in the absence of rhizobia, which requires transcription factors NSP1, NSP2, and NIN. NIN is one of the proteins of the plant-specific NLP family, and seems legume specific by its phylogenetic position. We screened genes whose expression was affected by the presence of NIN. Among them, we identified a set of genes that were necessary for bacterial infection in the epidermis and nodule development in the cortex. Those genes possessed specific nucleotide sequences in the upstream of ORF, to which NIN directly bound. Ectopic expression of the genes conferred aberrant formation of lateral roots. Our finding suggests that NIN has evolved to regulate formation of root nodules in legumes, by co-option of genes existed for lateral root formation.

CS17-2

Identification of a common regulator involved both in nodulation and shoot apical meristem maintenance in *Lotus japonicus*

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Nodulation is a characteristic trait acquired mainly by legume. Despite recent progress in our understanding of molecular mechanism regulating nodulation per se, its evolutionally genetic context that have enabled legume to form nodules remains elusive. In the early developmental process of nodulation, infection of rhizobia into the host plant root induces dedifferentiation and division of some of the cortical cells, and recent studies have clarified activation of cytokinin signaling in the cortical cells is a pivotal event for nodule organogenesis. To gain further insight into the molecular mechanism of it, we isolated *tricot* (*tco*) as a suppressor mutant of *spontaneous nodule formation2* (*snf2*), a gain-of-function mutant of cytokinin receptor in *Lotus japonicus*. In *tco snf2* double mutant, spontaneous nodules formed in *snf2* in the absence of rhizobia barely develop. The result suggests that *TCO* positively regulates nodule organogenesis downstream or independently of cytokinin signaling. In addition, infection process of rhizobia is affected and symbiosis with arbuscular mycorrhizal fungus is also impaired in *tco*. Intriguingly, the *tco* mutation causes an enlargement of the shoot apical meristem (SAM) and affects root development. Map-based cloning approach reveals TCO is a putative orthologue of the proteins that are reported to be involved in the SAM maintenance in other plants. These findings indicate an existence of a common genetic regulatory mechanism between nodulation and the SAM formation. We propose a hypothesis, in which an ancestor of legume might have recruited such gene regulating the SAM maintenance to achieve nodulation during its evolution.

CS17-3

Regulation of *Medicago truncatula* HMGR1 by symbiotic receptor-like kinases and its role in early symbiotic signaling

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HMGRs (3-hydroxy-3-methylglutaryl coenzyme A reductases) are key enzymes in the mevalonate pathway controlling isoprenoid biosynthesis. Surprisingly, one of these enzymes (HMGR1) was found to interact with the symbiotic receptor-like kinase NORK and is required for legume nodulation in the model legume *Medicago truncatula*. Using split-ubiquitin assays, interactions between HMGR1 and two other symbiotic receptor-like kinases, NFP and LYK3, were found. In vitro kinase assays revealed that HMGR1 is phosphorylated by NORK but not by NFP or LYK3. Mass spectrometry was used and localized the phosphorylation sites to the linker region of HMGR1, a region which is highly variable between different HMGR isoforms. Enzymatic assays revealed that HMGR1 activity is affected by interaction with NORK. Mimicking phosphorylation by serine to aspartic acid substitutions at the phosphorylation sites also affected HMGR1 activity. HMGR1-silenced roots were impaired for nuclear calcium spiking, symbiotic gene expression, and arbuscular mycorrhizal symbiosis, suggesting that HMGR1 is a component of the common symbiotic pathway. Reciprocally, application of mevalonate, the product of HMGR1 activity, was sufficient to induce calcium spiking and symbiotic gene expression in wild-type and HMGR1-silenced roots. These results indicate that HMGR1 plays an early role in the signaling cascade. We hypothesize that HMGR1 connects signaling events at the plasma membrane levels to nuclear ones by controlling the synthesis of isoprenoid compounds required for downstream symbiotic signaling.

CS17-4

New roles for strigolactones in legume symbioses

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There is currently much research being done to define new roles for the recently identified group of plant hormones, the strigolactones. One of their key roles is as regulators of plant symbioses. They act as a rhizosphere signal in arbuscular mycorrhizal symbioses and as a positive regulator of nodulation in legumes. Nutrient status of the soil has emerged as a powerful regulator of strigolactone production, most particularly phosphorous but also nitrogen. However, until now the potential role of strigolactones in regulating mycorrhizal development and nodulation in response to nutrient-deficiency has only been postulated but not tested. We critically examine the role of strigolactone synthesis and response in regulating both symbioses using pea (*Pisum sativum*), which has a range of well-characterised strigolactone-biosynthesis and response mutants that is unique amongst the legumes. We provide evidence for a novel endogenous role for strigolactone response within the root itself during mycorrhizal development, in addition to action of strigolactones on the fungal partner. We also reveal that the strigolactone response pathway that regulates mycorrhizal development may have some differences to the response pathway that regulates nodulation. Finally, studies with strigolactone-deficient pea mutants indicate that despite strong regulation of strigolactone production by both nitrate and phosphate starvation, strigolactones do not appear to be required to regulate these symbioses in response to nutrient-deficiency.

CS17-5

Intracellular accommodation of microbes by plants: Novel systems to study commonalities and differences between symbionts and pathogens

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Plants develop specialized cellular structures to engage with filamentous mi crobial organisms. For example, both oomycetes and arbuscular mycorrhiza (AM) fungi follow structurally similar developmental processes to establish intracellular interfaces, known as haustoria and arbuscules, respectively. Despite striking structural similarities, our knowledge of the molecular mechanisms that drive differentiation of host cells and tissues to form intracellular accommodation structures is limited. In particular, we lack plant systems that enable direct comparison of symbiotic and pathogenic interactions. Here we present new systems to study the extent to which beneficial and detrimental microorganisms employ similar plant developmental processes required for colonization. We employ the root-infecting oomycete *Phytophthora palmivora*, which infects Mycorrhiza-host plants such as *Medicago truncatula* as well as the model plant *Nicotiana benthamiana*. *P. palmivora* forms haustoria in *M. truncatula* roots which are analogous to Mycorrhiza arbuscular host-cell interfaces enabling comparative studies of interface processes.We exploited these systems in a variety of experiments. First, we used expression profiling to identify genes induced during both biotrophic *Phytophthora* infections and mycorrhization. Second, we tested an array of *M. truncatula* mutants defective in AM symbiosis and identified and characterised a mutant which impairs both AM colonisation and *P. palmivora* infection. Third, our systems enable comparative studies of effector targeted processes in AM fungal arbuscules. We found that perihaustorial *Phytophthora* effectors also localise to and interfere with fungal arbuscules.In summary, we expect these systems to greatly impact our understanding of commonalities and differences in beneficial and detrimental interactions between filamentous microbes and plant roots.

CS17-6

Essential factors for arbuscular mycorrhizal symbiosis: lessons from maize and rice

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The mutually beneficial arbuscular mycorrhizal (AM) symbiosis is the most widespread plant-fungal interaction between roots of terrestrial plants and fungi of the Glomeromycota. The association receives increasing scientific attention because of the nutritional benefit it confers to host plants, which is particularly pronounced for phosphate. Mutants defective in AM symbiosis resulted from a forward genetics screen in maize (PASZKOWSKI et al. 2006, Plant J. 47: 165-173). The *nope1 (no perception 1)* mutant displayed loss of susceptibility, indicative of pre-symbiotic function to be affected. The mutation segregated as a monogenic recessive trait and was mapped to the peri-centromeric region of maize chromosome 10. Gene cloning efforts employed a synteny-based approach in rice and identified a candidate gene, whose disruption reproduced the maize *nope1* phenotype, thereby suggesting the successful cloning of *NOPE1*. Insertion alleles in the corresponding maize gene have been identified via Ds tagging and are currently examined for their impact on symbiotic properties. The gene is predicted to encode a protein of unknown function but assumed to be involved in transport processes across membranes as it groups with the major facilitator superfamily. Recently, we have made the exiting observation that wild-type root exudates complemented the mutant phenotype in *trans*. It can therefore be hypothesized that NOPE1 participates in an efflux activity across the plasma membrane of root cells.

CS18-1

What does community analysis of plant-associated microbes tell us?

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Diverse microorganisms are living as endophytes in plant tissues and epiphytes on plant surfaces in nature. Questions about driving forces shaping the microbial community associated with plants remain unanswered. Because legumes developed systems to attain endosymbioses with rhizobia as well as mycorrhizae during their evolution, the above questions can be addressed using legume (soybean) and non-legume (rice) mutants relevant to plant genes for symbiosis. Analytical methods of microbial community have been recently advanced by enrichment procedures of plant-associated microbes and culture-independent analyses in microbial ecology. The global diversity of bacteria associated with field-grown soybeans was evaluated with different nodulation genotypes and nitrogen application. A subpopulation of Proteobacteria in soybean shoots was likely to be regulated through both of the autoregulation system for plant-rhizobium symbiosis and the nitrogen-signaling pathway, suggesting that legumes accommodate taxonomically characteristic microbial community through unknown plant-microbe communications. Impacts of *OsCCaMK* genotypes were examined on rice root-associated bacteria under paddy and upland field conditions. Phylogenetic compositions revealed that the relative abundance of Alphaproteobacteria was decreased in recessive plants under both paddy and upland conditions. Population shifts of Sphingomonadales and Rhizobiales were mainly responsible for the low abundance of Alphaproteobacteria in recessive plants. PCoA on bacterial communities revealed unidirectional community shifts in a manner of gene dosage effect for the functional *OsCCaMK*. These results suggest the significant impacts of *OsCCaMK* on the diversity of root-associated bacteria. Interestingly, the impacts were enhanced under an unfavorable environment of low N input, and extended to plant growth and geochemical processes.

CS18-2

Factors affecting endophytic colonization of rice

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Azoarcus sp. strain BH72, a mutualistic endophyte of rice and other grasses, is of agro-biotechnological interest because it supplies fixed nitrogen to its host and colonises plants in remarkably high numbers without eliciting disease symptoms. This raises the question of mechanisms of compatible interactions between host and bacterium. The complete genome of strain BH72 was sequenced (1), and the rice genome is also available. This allows application of functional genomic analyses of both partners during interaction. Transcriptomic analysis demonstrated that partners show extensive adaptations during endophytic interaction. Exudates-exposed *Azoarcus* sp. On exposure to exudates, an overall expression of 4.4% of the 3992 protein coding genes of *Azoarcus* sp. strain BH72 was altered, out of which 2.4% was up-regulated and 2.0% was down-regulated. Genes with modulated expression included a few whose involvement in plant-microbe interaction had already been established, whereas a large fraction comprised of genes encoding proteins with putative or unknown functions. Mutational analysis of several differentially regulated genes like those encoding a minor pilin PilX, signal transduction proteins containing GGDEF domains and a serine-threonine kinase as a putative component of the type 6 secretion system (T6SS), revealed their role in host colonization. Our data suggest that strain BH72 may be primed for the endophytic lifestyle by exudates, as the expression of bacterial genes relevant for endophytic colonization of roots is induced by root exudates.
(1) Krause et al. 2006. Genomic insights into the lifestyle of the mutualistic, N2-fixing grass endophyte *Azoarcus* sp. strain BH72. Nature Biotechnol. 24: 1385-1391.

CS18-3

Effects of colonization of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in *Arabidopsis*

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An endophytic bacterium, *Azospirillum* sp. B510, elicited systemic resistance against diseases caused by the virulent rice blast fungus *Magnaporthe oryzae* and by the virulent bacterial pathogen *Xanthomonas oryzae* in rice (*Oryza sativa* cv. Nipponbare). B510 confers disease resistance against the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) also in *Arabidopsis thaliana* ecotype Col-0. To investigate mechanisms underlying disease resistance, expression patterns of defense-related genes, *PR-1*, *PR-2*, *PR-5*, *PDF1.2*, *ERF1*, and *VSP2* and accumulation of defense-related phytohormones such as salicylic acid (SA) and jasmonic acid (JA) were investigated. SA-mediated *PR-1* genes expression was inhibited by treatment with B510, however, the contents of SA and JA were not changed. Treatment with B510 reduced pathogen proliferation in NahG transgenic plant and *etr1* and *jar1* mutants. However, bacterial growth in *npr1* and *ein2* mutants were not influenced by B510 treatment. Transcript levels of *PR-1* and *VSP2* genes after inoculation with *Pst* DC3000 were slightly increased in B510-treated plants compared to control plants. These results indicate the possibility that B510 primes NPR1- and EIN2-dependent disease resistance in *Arabidopsis*.

CS18-4

Genomic and transcriptomic analyses of the parasitic plants

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Parasitic plants are quite common and more than 4000 species are known to date. Among them, plants belonging to the family Orobanchaceae have emerged as serious threats in agriculture. For example, *Striga hermonthica*, the witchweed, is an obligate root parasite that infects economically important crops such as sorghum, maize, millet, and upland rice in sub-Saharan Africa, and the yield losses caused by this species have been estimated to cost as much as US$ 7 billion annually. Despite its agricultural importance, molecular mechanisms controlling the establishment of parasitism are poorly understood. To understand of the parasitism, we initiated large-scale genome and transcriptome analyses of *S. hermonthica* and its close relative *S. asiatica*. These analyses revealed an unexpected horizontal gene transfer event from the host to the parasite. We have also developed a model system to understand the parasitism using the hemiparasite *Phtheirospermum japonicum* belonging to Orobanchaceae. *P. japonicum* can be easily grown in the lab and is amenable for various genetic analyses, such as crossing, mapping and transformation. The transcriptome analysis has provided a list of genes specifically expressed during infection and a useful resource for molecular markers.

CS18-5

Reduced exudation of 5-deoxystrigol confers resistance to *Striga* in maize cultivars

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Witchweeds (*Striga* spp.), devastating root parasitic weeds, attack monocot crops including sorghum, millet, and maize in semi-arid tropics. Their seeds require germination stimulants (mainly strigolactones, SLs) released from host roots to germinate. In the present study, characterization of SLs in the root exudates from three maize cultivars, the *Striga*-susceptible Pioneer 3253, and the two *Striga*-tolerant KST94 and WH502, grown hydroponically was conducted by comparing retention times of germination stimulants on reversed-phase HPLC with those of synthetic and natural standards and by using LC-MS/MS. The most abundant SL in the root exudate from the susceptible cultivar was 5-deoxystrigol while the tolerant cultivars exuded mainly hydroxy-SLs such as strigol, sorgomol, and orobanchol. 5-Deoxystrigol is more stable than hydroxy-SLs and thus the susceptible cultivars would induce more germination of *Striga* seeds in the fields.

CS18-6

Nitrogen fluxes in the *Phelipanche ramosa* / *Brassica napus* interaction

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The holoparasitic plant *Phelipanche ramosa* L. Pomel (syn. *Orobanche ramosa*) strictly relies on nutrient uptakes from phloem elements of the host plants through a specific structure called haustorium. In France, its recent adaptation to winter oilseed rape (WOSR, *Brassica napus* L.) results in an emergent agronomical problem causing severe yield losses. Our study aimed to give a better understanding of some functional traits of this new host-parasite interaction. Given that fertilization plays a major role in WOSR productivity, our studies focused on nitrogen fluxes within host-parasite relationship. Using ^{15}N labeling, comparative analyses were performed between two WOSR accessions, ES Alienor (Seminis Company) and Shakira (Maisadour Semences Company), which induced rapid and delayed emergence of the attached parasites in fields, respectively. When challenged with *Phelipanche ramosa*, behaviour of Shakira is characterized by a lag in broomrape attachment and development. Two characteristics promote an important development of broomrape growing on ES Alienor : a higher susceptibility to *Phelipanche ramosa* before vernalization and an early important nitrogen flux from host leaves to parasite following vernalization. Analysis of free ^{15}N-aminoacid patterns in exsudates from WOSR leaf phloem and in broomrape organs give a better characterization of nitrogen fluxes within these interaction. Glu, Asp, Gln, S-MethylCysteine Sulfoxide “SMCSO”, Serine and GABA are mainly transferred by the phloem. SMCSO, Gln, Asp, Glu and Asn are mainly accumulated in broomrape. A study of the nitrogen metabolism of the host-derived aminoacids in broomrape is in progress.

CS19-1

Harpin, elicitor of hypersensitive response for new era agricultural application-opportunities and challenges

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Harpins are a group of naturally occurring proteins first isolated from Erwinia amylovora more than 20 years ago. Harpin proteins activate a plant's signaling mechanism by binding to a plant protein. This signaling mechanism turns on internal cascade responses in plants including the activation of two well-characterized plant defense pathways and further stimulates the expression of genes involved in plant growth, which results in a significant increase of marketable crop yield. After a decade of research and development driven by academic universities and private biotech companies, several harpin-derived products have been developed and commercialized. First-generation harpin products used a single natural harpin protein as the active ingredient, while the second-generation products are derived from a combination of active domains of individual natural harpin proteins, which ultimately results in higher potency and better performance. The primary applications of harpin products are seed treatment and foliar spray; either method can be used in combination with other chemical products such as fungicides. Currently harpin derived products have been widely adopted for use by the agricultural industry. Harpin products have been applied to millions of acres of various crops in many countries. This presentation will discuss the benefits of harpin products, its success and challenges in the marketplace.

CS19-2

Toward durable disease resistance to wheat rusts

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Two Blades Foundation supports the development of durable resistance to important crop diseases. As part of this effort, we work closely with The Sainsbury Laboratory. Our core strategy is based on identification of new resistance genes from previously untapped sources. For any disease target, we plan to clone many resistance genes and deploy them as stacks at single transgenic loci. One of our projects focuses on novel resistance genes effective against wheat stem and stripe rusts. We will present progress toward identifying and genetically characterizing new sources of resistance from several plant species.

CS19-3

Addition of TAL effector binding sites to a pathogen strain-specific rice bacterial blightresistance gene makes it effective against additional strains and against bacterial leaf streak

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Xanthomonas TAL effectors promote disease in plants by binding to and activating host susceptibility genes. Plants counter with TAL effector-activated executor resistance genes, which cause host cell death and block disease progression. We asked whether the functional specificity of an executor gene could be broadened by adding different TAL effector binding elements (EBEs) to it. We added six EBEs to the rice *Xa27* gene, which confers resistance to strains of the bacterial blight pathogen *X. oryzae* pv. *oryzae* (Xoo) that deliver TAL effector AvrXa27. The EBEs correspond to three other effectors from Xoo strain PXO99A and three from strain BLS256 of the bacterial leaf streak pathogen *X. oryzae* pv. *oryzicola* (Xoc). Stable integration into rice produced healthy lines exhibiting gene activation by each TAL effector, and resistance to PXO99A, a PXO99A derivative lacking AvrXa27, and BLS256, as well as two other Xoo and ten Xoc strains virulent toward wild-type *Xa27* plants. Transcripts initiated primarily at a common site regardless of activating effector. Sequences in the EBEs were found to occur nonrandomly in rice promoters, suggesting overlap with endogenous regulatory sequences. Thus, executor gene specificity can be broadened by adding EBEs, but caution is warranted due to the possible coincident introduction of endogenous regulatory elements.

CS19-4

Effector-driven disease resistance breeding in potato

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The major food crop potato severely suffers from the devastating late blight disease, which is caused by *Phytophthora infestans*. This oomycete pathogen secretes host-translocated RXLR effectors that include avirulence (AVR) proteins, which are targeted by resistance (R) proteins from wild potato species. We have generated a genome-wide infection-ready library of *P. infestans* RXLR effectors that we have been using to accelerate cloning and specificity profiling of *R* genes. This effectoromics strategy has proven effective and complementary to classical breeding approaches. We have identified and characterized approximately a dozen R-AVR pairs that can be immediately exploited to accelerate and improve late blight resistance breeding. Studies of effector diversity and activity revealed the mechanisms that *P. infestans* employs for evading R protein recognition for the various R-AVR pairs. Spatio-temporal monitoring of effector allelic diversity in *P. infestans* populations enables a more educated deployment of *R* genes in potato. Recently, we have expanded the R-AVR-based line of defense with studies on apoplastic immunity, which has generally a broader spectrum and is based on recognition of conserved proteins of pathogens. We have isolated a potato surface receptor ELR1 that senses elicitins, secreted oomycete proteins with features of pathogen-associated molecular patterns (PAMP). In transgenic potatoes, *ELR1* confers a hypersensitive response to INF1 elicitin of *P. infestans* and enhanced resistance to late blight. Our aim is to achieve effective and durable resistance against late blight in potato by combining multiple layers of immunity.

CS19-5

Application of MutMap to identify rice genes involved in blast resistance

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The majority of agronomic traits are controlled by multiple genes that cause minor phenotypic effects making gene isolation challenging. To isolate genes with minor effects by whole genome resequencng, we developed MutMap method. Following mutagenesis, a mutant with a useful phenotype is crossed to the original wild-type line allowing unequivocal segregation in the F2 progeny even of subtle phenotypic differences. Bulked DNA of 20 F2 progeny showing the mutant phenotype is subjected to whole genome resequencing. Scanning of the genome for regions exhibiting higher frequencies of sequence reads originating from the mutant identifies loci harboring the mutation. This method allows identification of mutated genes in a single run of whole genome

resequencing, circumventing development of DNA markers and reducing cost and effort in gene isolation. We are applying MutMap to isolate genes involved in rice blast resistance.

CS19-6

A polygalacturonase inhibitor confers to transgenic tobacco resistance against fungi and oomycetes

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We have tested whether a gene encoding a polygalacturonase-inhibiting protein of *Phaseolus vulgaris* L. (Pvpgip2) protects tobacco against a fungal disease (*Rhizoctonia solani*) and two oomycetes (*Phytophtora parasitica* var. *nicotianae* and *Peronospora hyoscyami* f. sp. *tabacina*) not only under greenhouse conditions but also in field trials. Under greenhouse conditions, disease symptoms caused by *R. solani* were severe on wild type plants and very limited on transgenic lines. Under greenhouse conditions transgenic tobacco was also remarkably resistant to the oomycete pathogen *P. parasitica* var. *nicotianae*. Trials were also conducted in the field during the cold and wet season when tobacco blue mold caused by *P. hyoscyami* f. sp. *tabacina* constitutes a significant problem in Cuba. Transgenic plants displayed a high level of resistance that was comparable to that of *Nicotiana* species that are naturally highly resistant to *P. hyoscyami* f. sp. *tabacina*. We concluded that expression of PGIP is a powerful way of engineering a broad-spectrum disease resistance. The transfer of a PGIP gene from common bean to tobacco, i.e. a plant belonging to the economically important class of Solanaceae, confers to transgenic plants a strong resistance against fungi and oomycetes, both in greenhouses and in the field. The structure of PGIPs is being studied in order to enlarge their recognition specificities and improve their inhibitory strength. This knowledge may help in planning mutational strategies aimed at improving the properties of the natural PGIPs and their recognition versatility against the many microbial PGs evolved in nature.

CS20-1

Life-style transitions in hemibiotrophic *Colletotrichum* fungi uncovered by comparative genome and transcriptome analyses

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Colletotrichum species are devastating pathogens on major crop plants worldwide. Infection involves differentiation of specialized cell-types associated with host penetration (appressoria), growth inside living host cells (biotrophic hyphae) and tissue destruction (necrotrophic hyphae). We sequenced and compared the genomes and transcriptomes of *C. higginsianum* (*Ch*) infecting Arabidopsis and *C. graminicola* (*Cg*) infecting maize. Both species encode large repertoires of carbohydrate-active enzymes but use different strategies to deconstruct plant cell walls that are adapted to their host preferences. Thus, *Ch* encodes more pectin-degrading enzymes and activates them during necrotrophy, while *Cg* predominately activates hemicellulases and cellulases at this stage. Both species encode more secondary metabolism (SM) key enzymes than most other sequenced fungi, with 42 SM gene clusters in *Cg* and 39 in *Ch*, suggesting each is capable of great chemical diversity. Genome-wide expression profiling revealed the transcriptional dynamics underlying hemibiotrophy, with waves of gene activation linked to each pathogenic transition. The early transcriptome is dominated by SM and effector genes, suggesting both appressoria and biotrophic hyphae function as platforms for delivering protein and small molecule effectors to the first infected cells. Genes encoding a vast array of wall-degrading enzymes, proteases and membrane transporters are up-regulated at the switch to necrotrophy, when the pathogen mobilizes nutrients from dead cells for growth and sporulation. Remarkably, although appressoria *in vitro* are morphologically indistinguishable from those *in planta*, comparison of their transcriptomes showed 1,500 genes are induced upon host contact, suggesting that pre-invasion sensing of plant signals by appressoria dramatically reprograms fungal gene expression.

CS20-2

Global reprogramming of DNA methylation during pathogenic development in the rice blast fungus

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A key determinant of microbial pathogenesis is an ability of pathogen to make morphological switching in response to environmental stimuli. This capacity to change form and structure is critical for survival, dispersal, and successful infection of hosts. Genetic pathways that regulate morphological transitions are extensively studied in many species of microbial pathogens, yet contribution of the epigenetic component is largely unknown. Here we use genetic manipulations and high-throughput bisulphite sequencing (methylC-seq) on the model plant pathogenic fungus, *Magnaporthe oryzae* to decipher the dynamics and mechanics of DNA methylation during fungal development at single-nucleotide resolution. We show that two genes encoding DNA methyltransferases are responsible in a cooperative fashion for DNA methylation in this fungus and that progression of fungal development correlates with genome-wide reduction and reprogramming of DNA methylome. Detailed analysis of methylC-seq data show that reduction and reprogramming is commonly associated with upstream and downstream regions of annotated genes, suggesting regulatory role of DNA methylation in transcription of genes. RNA-seq analysis of wild-type and DNA methyltransferase deletion mutants supports that transcript abundance of genes, transposable elements, and unannotated intergenic transcripts are altered by DNA methylation. Our works provide new insights into evolution of DNA methylation, revealing that DNA methylation in fungi is a dynamic epigenetic entity that may contribute to morphological switching driven by environmental cues.

CS20-3

Genomic evolution and specialization of wheat rust fungi

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Rust fungi cause some of the most devastating plant diseases on all major cereal crops. We sequenced the genomes of the three major rust pathogens of wheat: *Puccinia graminis* f. sp. *tritici* (*Pgt*, wheat stem rust or black rust pathogen), *Puccinia triticina* (*Pt*, wheat leaf rust or brown rust pathogen), and *Puccinia striiformis* f. sp. *tritici* (*Pst*, wheat stripe rust or yellow rust pathogen. These rust genomes are large compared to other fungi, ranging in size from 82 Mb to 106 Mb; all genomes contain a high fraction of repetitive sequence. Previous analysis of *Pgt* revealed features related to the obligate biotrophic life-style including a large repertoire of effector-like small secreted proteins (SSPs), impaired nitrogen and sulfur assimilation pathways, and expanded families of amino-acid, oligopeptide and hexose membrane transporters. Comparison of the three *Puccinia* genomes allows delineation of gene gain and losses both for the *Puccinia* as a group as well as differences between each species would could be important for different host adaptation and phenotypes. To gain insight into more recent evolution of virulence, we are comparing the sequence of isolates which vary in virulence phenotypes on wheat differentials, including 65 isolates of *Pt* and 56 isolates of *Pgt*, by identifying SNPs in these strains. Variation in sequence or gene expression of predicted secreted proteins can suggest candidate effector proteins for future study.

CS20-4

Evolution of cell entry function in oomycete and fungal effectors

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Symbionts, both pathogenic and beneficial, must integrate their physiology with that of their host in order to achieve a successful colonization. Effector proteins that enter the cytoplasm of host cells are widely utilized for this purpose by bacterial, fungal, oomycete, protistan, nematode, and insect symbionts. Thus mechanisms for

effector entry must have evolved numerous times among these diverse organisms. Here we will present recent progress on understanding how eukaryotic effectors enter host cells. The genomes of oomycete plant pathogens encode hundreds of potential effector proteins with the motif RXLR. RXLR domains are responsible for the entry of these proteins into plant cells. Domains with similar "RXLR-like" motifs appear to be responsible for entry by some effectors from some fungi and insects, including mutualistic fungi. RXLR- and RXLR-like-domain proteins from oomycetes, fungi and insects bind the cell surface lipid phosphatidylinositol-3-phosphate (PI3P) and this binding enables entry into the cells, possibly by endocytosis. In some effectors, additional residues in C-terminal domains also contribute to PI3P-binding, suggesting that these effectors have undergone "affinity maturation" to improve their binding to PI3P and thus their ability to effectively enter plant cells. We are currently exploring methodologies for disrupting PI-3-P-mediated effector entry in order to create new means for managing oomycete and fungal diseases and insect pests.

CS20-5

Mining the *Rhynchosporium commune* genome and transcriptome for pathogenicity determinants

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Rhynchosporium commune, formerly known as *R. secalis* is one of the most destructive pathogens of barley worldwide, especially in areas with cool temperate climates. It can lead to yield losses of up to 30-40 % and decrease in grain quality. Despite the damage that *R. commune* inflicts on barley crops, knowledge of its pathogenicity factors is almost non-existent. The challenge therefore is to gain a greater understanding of novel and essential pathogenicity determinants, as these represent good targets for recognition by host plant genotypes. Some pathogenicity determinants essential for the core biology of the pathogen during infection may also represent potential targets for new environmentally benign fungicides. Recent sequencing of *R. commune* germinated conidia transcriptome revealed enrichment for transcripts encoding potential structural cell wall proteins, adhesion proteins, plant cuticle and cell wall degrading enzymes, signalling proteins, stress response and detoxification enzymes, and nutrient transporters. A subset of transcripts encodes for small secreted proteins, representing putative effectors, including the well-characterised avirulence gene *Nip1*. *R. commune* genome and interaction transcriptome sequencing provided further information about the extent of gene families, as well as a subset of genes expressed at the onset of *R. commune* colonization of barley. Comparison of genome sequences from strains with different race specificities will allow rapid prediction of candidate effectors, including those less variable in *R. commune* populations. *R. commune* potential pathogenicity determinants will be prioritised for further functional analysis based on their expression profiles.

CS20-6

Identifying effector proteins in two fungal pathogens of *Brassica napus*

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Effectors are small secreted proteins (SSPs) produced by pathogens to modify or subvert defence responses of the host organism. *Leptosphaeria maculans*, a fungal pathogen of *Brassica napus* (canola), has 650 genes predicted to encode small secreted proteins (SSPs), and are potential effectors. The close relative *L. biglobosa* also infects Brassicas but has different symptomology, causing damaging stem cankers far less frequently. We aim to use this key difference of *L. biglobosa* to dissect genomic requirements for stem canker formation. The genome of *L. biglobosa* "canadensis" has been sequenced by an Illumina method, and compared to that of the published *L. maculans* reference genome. Compared to *L. maculans*, *L. biglobosa* has a relatively compact genome (30 Mbp) and lacks AT-rich, gene-poor repeats, however, both fungi have a similar number of predicted SSPs. ONDEX network analysis identified SSP ortholog clusters and revealed that few *Leptosphaeria* SSPs were present in both species. *L. biglobosa* SSPs specifically expressed during pathogenesis have been identified by RNAseq deep sequencing. Over 300 Leptosphaeria genes are specifically upregulated during growth in planta. Predicted SSPs are more likely than non-SSPs to have upregulated expression in planta, contributing 25% of the top 100 in planta upregulated genes but only 6% of the pathogen's gene content. Functional analysis of upregulated *Leptosphaeria* effectors is underway, including examples from the LysM chitin-binding family. *L. maculans* contains a gene with a four LysM domain structure that is absent in *L. biglobosa*, this conformation not been described in other pathogenic fungi.

CS21-1

Chitin-induced dimerization activates a plant immune receptor

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Pattern recognition receptors (PRRs) confer plant resistance to pathogen infection by recognizing the conserved pathogen-associated molecular patterns (PAMPs). The cell surface receptor Chitin Elicitor Receptor Kinase 1 of Arabidopsis (AtCERK1) directly binds chitin through its lysine motif (LysM)-containing ectodomain (AtCERK1-ECD) to activate immune responses. Our crystal structure of an AtCERK1-ECD complexed with a chitin pentamer reveals that their interaction is primarily mediated by a LysM and three chitin residues. By acting as a bivalent ligand, a chitin octamer induces AtCERK1-ECD dimerization that is inhibited by shorter chitin oligomers. A mutation attenuating chitin-induced AtCERK1-ECD dimerization or formation of non-productive AtCERK1 dimer by overexpression of AtCERK1-ECD compromises AtCERK1-mediated signaling in plant cells. Together, our data support the notion that chitin-induced AtCERK1 dimerization is critical for its activation.

CS21-2

Structural basis of dual R protein signalling in Arabidopsis

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A plant's ability to detect and resist the infection of a specific pathogen rests with two critical genes; a resistance (R) gene in the plant and a corresponding avirulence (effector) gene in the pathogen. The proteins products of R gene's play a surveillance role within the plant cell and stimulate defence signalling after recognition of a specific effector protein. The most predominant class of R genes encode tridomain proteins with a central nucleotide-binding (NB) domain, a C-terminal leucine rich repeat (LRR) and either a Toll-interleukin 1 receptor-like (TIR) domain or a coiled-coil (CC) domain at their N-terminus. Interestingly, resistance to some pathogen isolates require two NB-LRR proteins. An explicit example of this is presented in Arabidopsis where the TIR-NB-LRR proteins RPS4 and RRS1 are both required for resistance to three different pathogens. We have demonstrated that the TIR domain of RPS4 and RRS1 can form a direct and specific interaction *in vitro*, implicating a role for the TIR domains in coordinating dual resistance. In addition, we report crystal structures of both the RRS1 and RPS4 TIR domains, individually and in a heterodimer complex. The heterodimer structure reveals the interface that mediates the interaction between the RPS4 and RRS1 TIR domains. We are currently investigating mutations that disrupt this interaction and surveying any functional affects in an effort to understand the molecular basis of R protein mediated resistance signalling.

CS21-3

Crystal structure and interaction with host factors of the superfamily 1 helicase from *Tomato mosaic virus*

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Many plant positive-strand RNA viruses encode superfamily 1 (SF1) helicase domains. Although helicase domains play essential roles in viral RNA replication and other processes, crystal structures of viral SF1 helicases have not been determined. Herein, we report the crystal structure of a fragment encompassing the helicase domain of the replication protein from *Tomato mosaic virus* (ToMV-Hel). The structure reveals a novel N-terminal domain tightly associated with a helicase core. The helicase core contains two RecA-like α/β domains without any of the accessory domain insertions. The N-terminal domain contains a flexible loop, a long α-helix, and an anti-parallel six-stranded β-sheet. Prediction of secondary structures in other viral SF1 helicases and comparison of those structures with the ToMV-Hel structure suggested that many viral SF1helicases have a similar fold. On the basis of the structure, we constructed deletion mutants of ToMV-Hel and performed split-ubiquitin-based interaction assays in yeast to map which region interacts with TOM1 and ARL8, host proteins that are essential for tomato mosaic virus RNA replication. The results suggested that both TOM1 and ARL8 interact with the long α-helix in the N-terminal domain, and that TOM1 also interacts with the helicase core. Furthermore, location of previously characterized mutations in the helicase domains of tobamoviruses will be also discussed.

CS21-4

Structure-led studies of oomycete RXLR effectors: a conserved protein fold and new host targets

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In the absence of significant sequence conservation, structural biology offers unique opportunities to discover functional and evolutionary relationships in proteins. Oomycete phytopathogens including *Phytophthora* and *Hyaloperonospora* encode hundreds of modular effector proteins that are predicted to suppress host defence mechanisms and manipulate other cellular processes. These effectors can also be recognized by host resistance proteins, triggering a cell death response. Most RXLR-type effectors do not share significant sequence homology with other proteins making the functional annotation of virulence activities, the defining of evolutionary relationships and a molecular understanding of effector-triggered immunity a significant challenge. We have determined the structures of three *Phytophthora* RXLR effectors, AVR3a11, PexRD2 and PexRD16, which are unrelated in primary amino acid sequence. We discovered unexpected similarities in the folds of these proteins that suggest a common evolutionary origin. Intriguingly, this fold is also found in the *Hyaloperonospora* RXLR effector ATR1. We have used bioinformatics to predict this domain is widely conserved in phytopathogenic RXLR effectors. We propose that this protein fold may act as a stable scaffold, supporting functional diversification of effectors to develop and maintain new virulence activities, but also evade the plant immune system. Recently, we have used Y2H studies to identify plant host signaling proteins that interact with PexRD2. Using combined biochemical, structural and *in planta* approaches we are investigating the role of these host proteins in plant cell physiology. We are also exploiting our structure of PexRD2 to determine how the effector might manipulate the activity of these host cell targets.

CS21-5

Structural analysis of the flax-rust effector AvrM

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Plant immunity is usually triggered by the recognition of a pathogen effector protein by a plant resistance protein, leading to the activation of plant defenses, which often culminate in a localized cell death response. The R proteins can be divided into a few conserved families, while the effectors are diverse in both sequence and structure, and have roles in virulence and basal immune suppression. The flax rust effector AvrM is a secreted protein that is recognized by the M resistance protein in flax. AvrM is able to internalize into plant cells in the absence of the pathogen, and interacts directly with the M protein inside the plant cell. AvrM has no significant sequence similarity to proteins of known structure, and its virulence functions and cellular targets are unknown. We have determined crystal structures of two different variants of AvrM. One of these variants AvrM-A, is recognized by the M resistance protein, while the second variant avrM, is not detected by M and promotes disease. Both structures have a novel L-shaped helical fold and form a dimer with an unusual non-globular shape. Analysis of the N-terminal region important for cell entry revealed that several conserved hydrophobic residues are clustered together and surface exposed, and may be involved in mediating uptake of AvrM into the plant cell. Furthermore, comparison of the avrM and AvrM-A structures, and analysis of polymorphic residues combined with recent interaction studies suggest that a distinct surface region in AvrM mediates detection by the M resistance protein.

CS21-6

Protease-inhibitor arms-races in the tomato apoplast

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The immune response of tomato includes the production and secretion of several cysteine proteases (e.g. RCR3, PIP1 and C14) into the extracellular space, creating a proteolytic apoplast that is presumably harmful for colonizing pathogens. Tomato pathogens are secreting inhibitors that suppress the activities of these host proteases during infection. The fungal pathogen *Cladosporium fulvum* secretes AVR2, which inhibits PIP1 and RCR3 whereas the oomycete pathogen *Phytophthora infestans* secretes cystatin-like EPIC1 and EPIC2B, which inhibit RCR3, PIP1 and C14. RCR3 mediates AVR2 recognition in plants carrying the *Cf-2* resistance gene. In the absence of *Cf-2*, lack of RCR3 does not affect *C. fulvum* growth, whereas *PIP1* silencing causes hypersusceptibility, indicating that PIP1 is the operative target of AVR2 and RCR3 is a decoy. RCR3 is under diversifying selection in wild tomato, resulting in variant residues on the surface of the protease. These variant residues affect different protease-inhibitor interactions: one residue prevents AVR2 inhibition, whereas three others affect EPIC inhibition. Other variant residues affect the strength of HR. These studies reveal a relevant ongoing molecular arms-race in the tomato apoplast.

ABSTRACTS

Poster Sessions

PS01-001

Defence signaling triggered by flg22 and Harpin diverge at stilbenic biosynthesis in *Vitis* cells

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Plants can active defence to pathogen attack by two layers of innate immunity: pathogen-associated molecular pattern (PAMP) triggered immunity (PTI), or effector-triggered immunity (ETI), which often culminates in hypersensitive cell death. ETI can be triggered by the bacterial effector Harpin in suspension cells of the pathogen resistant grape *Vitis rupestris*, in contrast to the susceptible *Vitis vinifera* cultivar Pinot Noir. PTI can be activated by the bacterial PAMP flg22 in both cell lines. To get insight into the two modes defence signaling, we compared both lines after treatment with flg22 or Harpin. We found that extracellular alkalinisation was blocked by inhibition of calcium influx, and modulated by pharmacological manipulation of the cytoskeleton and mitogen-activated protein kinase activity with quantitative differences between cell lines and type of elicitor. In addition, an oxidative burst was detected that was much stronger and faster in response to Harpin as compared to flg22. In *V. rupestris*, both flg22 and Harpin induced transcripts of defence-related genes including *stilbene synthase*, microtubule disintegration and actin bundling in a similar way in *V. rupestris*, but differently in cv. Pinot Noir. In contrast to Harpin, flg22 failed to trigger significant levels of the stilbene *trans*-resveratrol, even in the highly responsive *V. rupestris*. We discuss these data in a model, where PAMP flg22- and effector Harpin-triggered defence responses overlap in their early signaling, but diverge at stilbene biosynthesis, leading to a qualitatively different final response.

PS01-002

Is Peptidoglycan recognized in plants via a LysM-protein receptor complex?

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MAMP (microbe-associated molecular pattern) recognition systems play a key role in the plant innate immunity. Peptidoglycans (PGN), which are one of the major components of bacterial cell walls, are typical MAMPs inducing innate immune responses in the model plant *Arabidopsis thaliana* (1). To identify the corresponding PGN pattern recognition receptor(s) in Arabidopsis we focused on the LysM domain proteins that have been widely implicated in the recognition of GlcNAc-containing glycans. For example, lipochitooligosaccharide Nod-factors and chitin are recognized by the LysM domain contain receptors, NFR1/5 and CEBiP/CERK1, respectively. We recently identified three Arabidopsis LysM domain receptor-like proteins, LYM1, LYM3 and CERK1, that are involved in the PGN perception (2). The plasma membrane-tethered proteins LYM1/LYM3 physically bind to PGN, whereas CERK1 does not but is likely required for signal transmission across the plasma membrane. This system is analogous to *Os*CEBiP/*Os*CERK1-mediated chitin perception and immune activation in rice. *Os*CEBiP and *Os*CERK1 directly interact in a ligand-dependend manner (3). We are now analyzing if LYM1/LYM3 and CERK1 also physically interact to form a functional PGN-recognition complex in vitro (yeast-two-hybrid system, far western analysis) and in vivo (co-immunoprecipitation). (1) Gust, AA. et al., JBC, 282, 32338 (2007); (2) Willmann, R. et al., PNAS, 108, 19824 (2011); (3) Shimizu, T. et al., Plant J, 64, 204 (2010).

PS01-003

The role of antisense transcription in the quorum sensing regulation in *Pectobacterium atrosepticum* SCRI1043

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The transcriptome profile of bacteria can be extremely complex. In addition to mRNAs, rRNAs, tRNAs and regulatory small RNAs, a significant amount of *cis*-encoded antisense transcripts have been revealed. Some of them can be produced due to transcription of the overlapping genes towards each other. Although the portion of such convergently transcribed genes in bacterial genomes reaches more than 10%, the physiological role of this phenomenon remains unclear. In several species such as *Pectobacterium atrosepticum* (*Pba*), *Pseudomonas syringae*, *Serratia marcescens* and *Pantoea stewartii*, quorum sensing-related genes which encode LuxI and LuxR homologues are convergently transcribed and two open reading frames partially overlap. In *Pba expI* gene encodes the synthase of quorum sensing pheromone acylhomoserine lacton (AHL) and *expR* gene encodes AHL sensor. To elucidate whether the topology of these genes has a regulatory role we transferred the *expI-expR* loci from *Pba* into *Escherichia coli*. Both genes were placed under inducible promoters. Additionally, *expR* sequence was modified to prevent protein synthesis. The obtained model allowed us to assess the effect of synthesis of *expR* transcripts on the expression of the *expI* gene. Our data demonstrated that depending on the level of transcriptional activity *expR* acts as either weak activator or strong repressor of AHL production. Furthermore we determined that relative abundance of *expI* and *expR* mRNAs and their antisense RNAs in the *Pba* cells exhibited dependence on growth conditions. The obtained data demonstrate that in *Pba* antisense transcription is involved to the quorum sensing regulation.

PS01-004

Revealing mechanisms underlying conserved MLA-mediated immunity in monocots and dicots by interfamily gene transfer

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In plants and animals, the NLR family perceives non-self and modified-self molecules inside cells and mediates immune responses to pathogens. Immunity mediated by this receptor class is believed to be species-specific and it has been suggested that the function is restricted to closely related plants. The plant kingdom can be divided into monocots and dicots, which were separated by evolution ~150 million years ago. It is thus surprising that a NLR receptor (MLA) from monocotyledonous barley is fully functional in dicotyledonous *Arabidopsis thaliana*. We introduced *MLA* gene constructs in a partially immunocompromised *A. thaliana* background, because wild-type *Arabidopsis* is resistant to the barley powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*). Reminiscent of the MLA-triggered immune response in barley, immunity against *Bgh* in MLA-expressing stable transgenic *Arabidopsis* plants is specifically detected upon challenge with an avirulent *Bgh* strain. This immune response is associated with a host cell death response at the infection site as in barley. Thus, MLA-mediated immunity in *Arabidopsis* to *Bgh* is an authentic strain-specific resistance response. These data imply that monocots and dicots, despite their long evolutionary separation, still follow a common principle of immune mechanism. Recently we identified a minimal signaling module of the MLA receptor and resolved its crystal structure. We utilize this information to elucidate the signal initiation and transduction through this module. We present genetic and molecular data underlying MLA-mediated immunity in *Arabidopsis* and compare these with MLA activity in barley to

identify conserved receptor targets and signaling pathways across plant linages.

PS01-005

Analysis of the Defensome complex in rice innate immunity

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We have previously shown that OsRac1, a small GTPase in rice, plays critical roles in reactive oxygen species production, defense gene activation and initiation of cell death during defense responses. Recently, we have tried to isolate OsRac1-interacting proteins by various methods. Our current efforts revealed the signaling network of OsRac1, named the Defensome network, which is composed of immune receptors, molecular chaperones, scaffold proteins, OsRac1 and OsRac1 effectors. Here, gel filtration experiments showed that OsRac1 formed two different sizes of complex, a low (30-60 kDa) and a high (200-400 kDa) molecular weight OsRac1 complexes. We named the high molecular weight OsRac1 complex the "Defensome complex" and investigated its component and dynamics of formation. We found that the Defensome complex is composed of HSP90, HSP70, the molecular chaperone Hop/Sti1a, the chitin receptor OsCERK1, and the R protein Pit. Interestingly, co-immunoprecipitation experiments revealed that OsCERK1 and Pit are not present together in the Defensome complex. These results suggest that the Defensome complex forms two types of complex: one is the MTI-Defensome complex containing a MAMP receptor like OsCERK1 and the other is the ETI-Defensome complex containing an R protein like Pit. Thus, Defensome complexes work as key regulators in rice innate immunity.

PS01-006

Disruption of sphingolipid biosynthesis in *Nicotiana benthamiana* activates salicylic acid-dependent responses and compromises resistance to *Alternaria alternata f. sp. lycopersici*

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Sphingolipids play an important role in signal transduction pathways that regulate physiological functions and stress responses in eukaryotes. In plants, sphingolipids are important components in the defense response against bacterial and fungal pathogens. In fact, the virulence of two unrelated necrotrophic fungi, *Fusarium verticillioides* and *Alternaria alternata* which are pathogens of maize and tomato plants, respectively, depends on the production of sphinganine analog mycotoxins (SAM). These metabolites inhibit *de novo* synthesis of sphingolipids in their hosts to cause accumulation of long-chain bases (LCB) which are key regulators of programmed cell death (PCD). To gain more insight into the function of sphingolipids in plant immunity, we disrupted sphingolipid metabolism in *Nicotiana benthamiana* through the silencing of the serine palmitoyltransfersase (SPT), which catalyzes the first reaction in LCB synthesis. Efficient silencing of SPT was achieved and it profoundly affected plant development as it caused growth reduction and morphological changes in leaves and flowers. It also altered sphingolipid composition, as the total levels of phytosphingosine decreased while sphinganine and sphingosine levels increased, compared with control plants. Moreover, SPT-silencing compromised *N. benthamiana* resistance against *A. alternata*, which was associated with accumulation of salicylic acid (SA) and constitutive expression of the SA-induced *NbPR-1* gene. Exogenous sphinganine and fumonisin B1, a SAM produced by *F. verticillioides*, also up-regulated *PR-1* expression in *N. benthamiana* wild-type seedlings. Our results strongly suggest that LCB are novel modulators of the SA-dependent responses and provide a working model on the potential role of SAMs in disrupting the plant host response.

PS01-007

Reduction of sphingolipid 2-hydroxy fatty acids has an impact on defense response through decrease of membrane rafts in rice

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Plants have highly and sophisticated innate immunity system to defend themselves against a variety of biotic stresses. It has been reported that several proteins including some receptor-like kinases or OsRac1, one of the small GTPases which is important in rice innate immunity, may exist on microdomains in plasma membrane (PM), or membrane rafts. Membrane rafts are small, heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains, and help protein-protein and protein-lipid interactions to activate cellular reactions such as defense responses. However, it remains unclear whether membrane rafts affect the mechanism of plant innate immunity, and if so, how membrane rafts regulate defense responses. Then, we tried to investigate the relationship between rafts and rice innate immunity by modifying 2-hydroxy fatty acids of sphingolipids that are reported to contribute to the raft formation *in vitro*. There are two genes encoding sphingolipid fatty acid 2-hydroxylase (OsFAH1 and OsFAH2) in rice, and we established knock-down lines (OsFAH-KD) by the RNAi system. GC-MS analysis showed that 2-hydroxy fatty acids of sphingolipids were substantially reduced in OsFAH-KD. In addition, phase of PM was disordered in OsFAH-KD when membrane order was visualized *in vivo*, implying that sphingolipid 2-hydroxy fatty acid serves as a main factor that composes rafts in plant cells. Moreover, the expression of defense-related genes, such as *PAL1* and *PBZ1*, were abnormal in the treatment of chitin elicitor in OsFAH-KD. These results suggest that reduction of membrane rafts by deletion of sphingolipid 2-hydroxy fatty acids affects the mechanism of rice innate immunity.

PS01-008

A MACPF protein is required for cell death regulation in biosynthesis of antifungal compounds in *Arabidopsis*

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Arabidopsis thaliana exhibits durable resistance, called nonhost resistance, against non-adapted *Colletotrichum* species that are the causal agents of anthracnose disease. We previously isolated Arabidopsis *lic1* mutants (lesion induced by nonadapted *Colletotrichum*). The *lic1*-mediated cell death was not accompanied by invasion of non-adapted *Colletotrichum* species, suggesting that *LIC1* is involved in cell death regulation in nonhost resistance. Positional cloning of *LIC1* revealed that *LIC1* is allelic to *NSL1* encoding a protein with a MACPF domain. In contrast to the *lic1* mutants, the *nsl1* mutants, tagged by Ds transposon, is dwarf with spotted necrotic lesions in the absence of pathogen. *PEN2* encodes a myrosinase involved in glucosinolate metabolism for antifungal defense. Surprisingly, the *pen2* mutation suppressed the *lic1* phenotype, suggesting a link between PEN2-mediated antimicrobial response and LIC1/NSL1. In contrast, the *pad3* mutation only has slight suppression effects on the *lic1* phenotype. Mammalian MACPF proteins are involved in pore-formation on plasma membranes of target cells in immune responses. Expression of GFP-LIC1 suggested that LIC1 is targeted to plant plasma membrane. These findings suggested a possible involvement of LIC1/NSL1 in export of the PEN2-related metabolites. Consistently, genetic

inactivation of the PEN3 ABC transporter, involved in the export of the PEN2-related metabolites, enhanced the *lic1* phenotype. It was reported that flg22-triggered callose formation depends on PEN2 and PEN3, suggesting the requirement of the PEN3-dependent exportation. In contrast, flg22 induced callose deposition in the *lic1* mutants, suggesting that LIC1/NSL1 is not essential for the export of the PEN2-related metabolites in the flg22 treatment.

PS01-009

FMO1 and ALD1 mediate a common NPR1-dependent and SA-independent defence signal

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The Arabidopsis lesion-mimic double mutant *pen1 syp122* exhibits multiple activated defence signalling pathways in the absence of pathogens. In a previous suppressor mutant screen, ALD1 (AGD2-LIKE DEFENSE RESPONSE PROTEIN) and FMO1 (FLAVIN-DEPENDENT MONOOXYGENASE 1) were discovered to play important roles in defence related lesion formation and plant growth retardation (1). FMO1 and ALD1 have previously been demonstrated to have important roles in pathogen defence. Although it is known that FMO proteins catalyse the transfer of hydroxyl groups to nucleophilic heteroatom-containing substrates such as sulphur, nitrogen, selenium, or iodine, the specific substrate and product of FMO1 remain unidentified. Furthermore, FMOs can change the cellular redox state through the production of reactive oxygen species. ALD1 is suggested to have aminotransferase activity, and could be involved in lysine degradation. In the present study, rosette leave size analysis of triple, quadruple and quintuple mutants in the *pen1 syp122* background suggests that ALD1 and FMO1 act on the same defence signalling pathway, which is independent of SA-signalling, but dependent on the SA-downstream component, NPR1.
(1) Zhang Z, Lenk A, Andersson MX, Gjetting T, Pedersen C, Nielsen ME, Newman M-A, Hou B-H, Somerville SC, Thordal-Christensen H (2008). A lesion-mimic syntaxin double mutant in *Arabidopsis* reveals novel complexity of pathogen defense signalling. *Molecular Plant* 1, 510-527.

PS01-010

Development of Raichu FRET sensors to monitor the immune responses in *Arabidopsis thaliana*

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Plants have a unique subfamily of Rho-family GTPases, called Rops (Rho-related GTPase from plants) or Racs. Rac/Rop family members have been found in most all studied species. Recent studies have implicated Rac/Rop signaling in diverse processes ranging from cytoskeletal organization to hormone and immune responses and their cellular targets are predominantly the actin cytoskeleton, cytosolic Ca^{2+} concentration and reactive oxygen species (ROS) production. Raichu (Ras and interacting protein chimeric unit) sensor was developed to monitor the local activity of Rho family GTPase. We have previously developed Raichu-OsRac1 for monitoring OsRac1 activation in rice. This sensor is based on fluorescence resonance energy transfer (FRET) and consists of OsRac1, the Cdc42/Rac interactive binding (CRIB) motif, YFP and CFP. Upon activation of OsRac1, the binding to CRIB increases the efficiency of FRET between CFP and YFP. Raichu-OsRac1 enables imaging and quantification of the spatio-temporal activation of OsRac1 in live cells. Using Raichu-OsRac1 sensor, we demonstrated that OsRac1 is activated by MAMPs (Microbe-Associated Molecular Patterns) and R proteins in rice protoplasts. These results showed a decisive role of Rops in plant disease resistance. However, most of these studies have been only conducted in rice. Here, to reveal the function of Arabidopsis ROPs (AtROPs) in immune responses, we developed Raichu-AtROPs, which are derived from Raichu-Rab5 and applied to study activation of AtROPs in immune responses. We show that AtROPs act early in chitin signaling pathway of *Arabidopsis thaliana*.

PS01-011

NbMIP1, a J-domain Protein, is required for both *Tobacco mosaic virus* infection and plant disease resistance

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Tm2² is a CC-NBS-LRR resistance gene and confers the durable and extreme resistance against *Tomato mosaic virus* (ToMV) and *Tobacco mosaic virus* (TMV) by recognizing the presence of viral movement protein (MP). Here we report that NbMIP1, a novel J-domain protein, associates with both TMV MP and Tm2² in vitro and in vivo. Suppression of *NbMIP1* in *N. benthamiana* plants suggests that it has a role in plant development. Further, silencing of *NbMIP1* in *N. benthamiana* plants reduces the TMV cell-to-cell movement. In addition, *NbMIP1* suppression in *Tm2²*-containing plants compromises *Tm2²*-mediated resistance to ToMV and TMV. We found that silencing of *NbMIP1* reduces the steady-state protein levels of TMV MP and Tm2². These results suggest that *NbMIP1* is required not only for virus infection but also for *Tm2²*-mediated virus resistance by maintaining the steady-state levels of proteins.

PS01-012

The *Magnaporthe oryzae* effectors AvrCO39 and Avr-Pia are recognized by the rice Nucleotide Binding-Leucine rich repeat (NB-LRR) protein RGA5 through direct interaction

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Plant immunity strongly relies on direct or indirect recognition of pathogen effectors by plant resistance (R) proteins. This recognition activates disease-resistance signaling pathways leading to the inhibition of pathogen growth and the induction of a localized programmed cell death called the hypersensitive response (HR). To gain a better understanding of the molecular mechanisms governing effector recognition in plants, we study two translocated effectors from the rice blast fungus *Magnaporthe oryzae*: Avr-Pia and AvrCO39. Our work shows that both sequence-unrelated effectors are recognized by the same duo of rice NB-LRR proteins, RGA4 and RGA5. *RGA4* and *RGA5* genes are located next to each other on rice chromosome 11 and are both necessary to confer resistance to *M. oryzae* strains expressing either Avr-Pia or AvrCO39. Interestingly, *RGA5* transcripts are alternatively spliced leading to the production of two protein variants termed RGA5-A and RGA5-B. Yeast two hybrid analysis revealed that Avr-Pia physically and specifically interacts with RGA5-A via a small RGA5-A specific domain whereas AvrCO39 interacts with RGA5-B via another small RGA5-B specific domain. This suggests that RGA5-A and RGA5-B act as receptors mediating specific recognition of the effectors by direct binding while RGA4 might act as a signaling component activating downstream resistance pathways. Furthermore, these results indicate that alternative splicing might be a mechanism contributing to the evolution and diversification of plant R-gene repertoires. Recent advance in the investigation of RGA5-A and -B recognition specificities and in the validation of the observed interactions will be presented.

PS01-013

Lipid modification of the NB-LRR-type R protein Pit is required for its localization to the plasma membrane and immune responses

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The nucleotide-binding domain and leucine-rich repeat (NB-LRR)-containing family proteins function as intracellular immune sensors in both plants and animals. NB-LRR family proteins recognize pathogen-derived molecules directly or indirectly and trigger a variety of immune responses. In plants, the molecules activated by NB-LRR family proteins and the mechanism of immune response induction by these downstream molecules are largely unknown. We have recently found that the small GTPase OsRac1 is activated by Pit, an NB-LRR-type R protein, and this activation plays a critical role in R protein-mediated immunity in rice. However, the sites and mechanism of Pit activation in vivo are largely unknown. To elucidate the mechanisms involved in the localization of Pit, we searched consensus sequences in Pit for membrane localization and found 1 potential palmitoylation site. Wild-type Pit was localized mainly on the plasma membrane, and this membrane localization was compromised in the palmitoylation-deficient mutant of the protein. The active form of Pit induced a hypersensitive response and reactive oxygen species production, whereas the palmitoylation-deficient Pit failed to induce both responses. The interaction of the palmitoylation-deficient Pit with OsRac1 on the plasma membrane was significantly lower than that of wild-type Pit. Furthermore, in vivo Forster resonance energy transfer experiments indicated that the active form of Pit induced the activation of OsRac1 on the plasma membrane. These results suggest that palmitoylation of Pit is important for its localization and interaction with OsRac1 on the plasma membrane and may play an essential role in the activation of OsRac1.

PS01-014

Plant immune receptors: what are the first steps that trigger defence signalling?

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Plant disease resistance can be triggered by specific recognition of microbial effectors by plant nucleotide binding leucine rich repeat (NB-LRR) receptors. However, the mechanisms controlling NB-LRRs activation and signalling are poorly understood. In flax, the L6 protein is a Toll/interleukin-1 receptor (TIR) containing NB-LRR which confers resistance to the flax rust fungus (*Melampsora lini*) containing the AvrL567 effector. Using a structure-function analysis approach, we previously demonstrated that L6 activation depends on the dimerization of its signalling TIR domain (Bernoux et al., 2011). To further define the L6 activation model, two questions have been investigated: i) how L6 activity is regulated before pathogen perception and ii) where/how the early signalling steps following L6 TIR dimerization are triggered.i) In the absence of a pathogen ligand, NB-LRRs have to be kept in an inactive state to avoid inappropriate defence activation and cell death. By using structure-guided mutagenesis and L allele comparisons, we identify two regions in L6 that may be involved in intramolecular interactions and control the inactive to active state equilibrium. ii) L6 is attached to the Golgi membrane through its N-terminal signal anchor (Takemoto et al, 2012). A subcellular localisation study of the L6 TIR signalling domain revealed that its membrane attachment is required to induce downstream defence signalling.

PS01-015

The CERK1-RacGEF-OsRac1 pathway is involved in chitin-induced immunity in rice

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In plants, perception of microbe-associated molecular patterns (MAMPs) with receptor-like kinase triggers innate immune responses. Previous studies have shown that the small GTPase OsRac1, belonging to the Rac/Rop GTPase family, is a key regulator in the MAMP-triggered immunity (MTI) pathway in rice. However, the spatio-temporal dynamics of transmitting the signal to the OsRac1 from the receptor-like kinases during the MTI pathway is unknown. Here we report that N-acetylchitooligosaccharide (chitin) elicitor, a MAMP derived from the rice blast fungus, induced rapid OsRac1 activation at the plasma membrane of rice protoplasts. We detected activation using Raichu-OsRac1, an intracellular Forster resonance energy transfer (FRET) biosensor that facilitates the in vivo monitoring of OsRac1 activation by elicitors. Moreover, we identify that a guanine nucleotide exchange factor (GEF) against OsRac1, which we termed OsRacGEF1, by yeast two-hybrid screening. It is established that a class of plant-specific RopGEFs promotes the activity of Rop/Rac through the catalytic PRONE (Plant-specific Rop nucleotide exchanger) domain. Our data show that OsRacGEF1 activates OsRac1 by exchanging GDP for GTP in vivo and in vitro, and interacts with OsRac1 at the plasma membrane. In addition, our data indicate that the OsRacGEF1 interacts with the receptor-like kinase OsCERK1, which was identified as a chitin receptor having a LysM motif in its the extracellular domain, and is phospholylated by intracellular domain of OsCERK1. These results indicate that the recognition of N-acetylchitooligosaccharide elicitor by OsCERK1 induces OsRacGEF1 activation by phosphorylation, and activated OsRacGEF1 then facilitates OsRac1 activation, at an early step in rice innate immunity.

PS01-016

The Gac-Rsm and SadB signal transduction pathways converge on AlgU to repress flagellar synthesis in the rhizobacterium *Pseudomonas fluorescens* F113

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Flagella mediated motility, an important trait for competitive rhizosphere colonization and biocontrol ability, is tightly regulated in *Pseudomonas fluorescens* F113. We have previously shown that swimming motility is repressed independently by the GacA/GacS system and by SadB through downregulation of the *fleQ* gene, encoding the master regulator of the synthesis of flagellar components. Here we show that both regulatory pathways converge in the regulation of transcription and possibly translation of the *algU* gene, which encodes a sigma factor. AlgU is required for multiple functions, including the expression of the *amrZ* gene which encodes a transcriptional repressor of *fleQ*. Gac regulation of *algU* occurs during exponential growth and is exerted through the RNA binding proteins RsmA and RsmE but not RsmI. RNA immunoprecipitation assays have shown that the RsmA protein binds to a polycistronic mRNA encoding *algU*, *mucA*, *mucB* and *mucD*, resulting in lower levels of *algU*. We propose a model for repression of the synthesis of the flagellar apparatus linking extracellular and intracellular signalling with the levels of AlgU and a new physiological role for the Gac system in the downregulation of flagella biosynthesis

during exponential growth.

PS01-017

SUMO-mediated transcriptional reprogramming in plant stress and innate immune responses

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Critical for an effective plant innate immune response is the ability of a plant to rapidly induce transcriptional reprogramming in response to pathogen invasion. In non-infected plants this defense response is suppressed by the SUMO (small ubiquitin-like modifier) machinery including the SUMO E3 ligase SIZ1. Correspondingly, SUMO mutants show constitutive SA-dependent defense activation. SUMO (small ubiquitin-like modifier) is a protein modification that modulates the activity and the recruitment of chromatin-modifying enzymes and transcriptional co-adaptors to transcription sites. While stress conditions induce sumoylation of many proteins including heat shock proteins, SUMO is deconjugated from transcription factors. We are using transcriptomics to reveal how SUMO controls gene regulation in response to heat stress and defense signaling in the model plant *Arabidopsis thaliana*. In addition, we are building a SUMO protein network based on yeast two-hybrid interactions, *in vitro* SUMOylation assays, and SUMO proteomics. This should reveal SUMO-dependent transcription regulation hubs and the role of SUMO in stress and defense signaling via these hubs. This network will be also used to examine the role of the "non-conserved" SUMO paralogs.

PS01-018

The *Arabidopsis* endogenous elicitor/receptor Pep/PEPR pathway links different branches of plant immunity

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Recognition of molecular structures typical of a microbial class, designated microbe-associated molecular patterns (MAMPs), leads to the so-called MAMP-triggered immunity (MTI) that restricts the invasion and growth of pathogenic microbes. MTI activation is also linked to confer systemic acquired resistance (SAR). However, the mechanisms that couple MAMP recognition to robust immune activation remain poorly understood.The *Arabidopsis* Leu-rich repeat receptor kinases PEPR1 and PEPR2 recognize the Pep-epitopes of PROPEP1-PROPEP6, triggering an immune response that is reminiscent of MTI.Of the six *PROPEP* genes, *PROPEP2* and *PROPEP3* are massively upregulated upon pathogen-derived elicitors, suggesting a role of the Pep/PEPR pathway in the amplification and/or spread of defense signaling. However, how the PEPR pathway contributes to host immunity remains elusive. Here we show that basal defense against hemibiotrophic pathogens and SAR are compromised in *pepr1 pepr2* plants, providing evidence for a role of this signaling system in plant immunity. To gain insight into the underlying mechanisms for Pep/PEPR-triggered immunity, we performed genome-wide transcriptome analysis. This revealed commonalities and differences between EFR- and PEPR-regulated genes and pathways. Our data indicate that Pep/PEPR signaling activation facilitates co-activation of the salicylate and jasmonate pathways that would otherwise typically antagonize each other, consistent with a role of PEPRs for defenses against hemibiotrophic pathogens. We propose a model in which the Pep/PEPR pathway links different cell autonomous and non-cell autonomous branches in plant immunity.

PS01-019

Ethylene and endogenous elicitor/receptor signalling serve at a post-recognition step in MAMP-triggered immunity

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Recognition of microbe-associated molecular patterns (MAMPs), conserved structures typical of a microbial class, triggers immune response that restricts microbial invasion and growth. However, the molecular basis of MAMP-triggered immunity (MTI) is largely unknown. In *Arabidopsis*, the Leu-rich-repeat receptor kinases (LRR-RKs) FLS2 and EFR recognize the bacterial MAMPs flagellin and EF-Tu (and their bioactive epitopes flg22 and elf18), respectively. Likewise, the LRR-RKs PEPR1 and PEPR2 recognize the endogenous elicitor epitopes Peps derived from the PROPEP family. We revealed *priority in sweet life6* (*psl6*) mutants that are impaired in several flg22- and elf18-triggered outputs and exhibit enhanced susceptibility to *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*). *PSL6* identifies a novel allele of *EIN2* encoding the master regulator of ethylene signaling. In contrast to a great decrease of *FLS2* expression, *EFR* expression and stable receptor accumulation are retained in *ein2* plants. Genome-wide transcriptome profiling revealed an inventory of EFR-regulated genes that are modulated by EIN2. This indicates a role of EIN2 for activation of a subset of SA-responsive genes and for suppression of a MYC2-dependent JA-branch. Indeed, EFR-triggered immunity is reduced in *ein2* plants towards a *Pst* mutant strain devoid of coronatine, which acts through the host MYC2-JA branch for virulence promotion. Moreover, our data also point to a contribution of the PEPR pathway to EFR-triggered immunity in both ethylene-dependent and independent manners. We propose the existence of different branches emanating from the receptor that differentially engage ethylene and PEPRs in MTI.

PS01-020

Identification of PTI signaling components through a suppressor screen using the novel allele *bak1-5*

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Plants utilize surface-localized receptors to sense microbial proteins and trigger an early immune response known as pathogen-associated molecular pattern (PAMP) triggered immunity (PTI). The short leucine-rich repeat receptor-like kinase (LRR-RLK) BAK1 is a master positive regulator of PTI and is important for plant defense. BAK1 was initially identified as an interactor and positive regulator of the brassinosteroid (BR) receptor BRI1, but also forms ligand-induced complexes with PAMP receptors, such as the LRR-RLKs FLS2 and EFR. Because of the multi-functionality of BAK1, a clear conclusion about the role of BAK1 in immunity has been hampered by the pleiotropic phenotypes of bak1 mutants linked to hypo-responsiveness to BR and increased cell death. We recently identified a novel allele, bak1-5, that is strongly and specifically impaired in PTI, but is fully functional in BR signalling and cell death control (1). Taking advantage of these unique properties, we carried out a bak1-5 suppressor screen and identified 11 modifier of bak1-5 (mob) mutants that restore PAMP-induced ROS burst in the bak1-5 background. Further analysis showed that the mob mutants restore additional immunity phenotypes including seedling growth inhibition, MAPK activation, and resistance to the hemi-biotrophic bacterium *Pseudomonas syringae* pv. *tomato* DC3000. To uncover causal mutations, we are combining positional cloning with whole-genome re-sequencing by screening through F2 populations from back- and wide-crosses. The identification of mob loci will add significantly to our understanding of immunity in plants.

(1) Schwessinger et al. PLoS Genet (2011) 7(4): e1002046.

PS01-021

Arabidopsis NIFC1, a component of SCF E3 ligase, implies to act as a negative regulator in plant immunity

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Ubiquitin Proteasome System (UPS) plays crucial roles during the whole course of plant growth events, including cell development, signal transduction, metabolic regulation, plant immunity and the others. The UPS can recognize and degrade short-lived regulatory proteins as well as abnormal or misfolded proteins induced by biotic and abiotic stresses. To clarify the processes involved in plant immunity, we characterized *Arabidopsis nsl2* (*necrotic spotted lesion2*) mutant, which has been originally reported as the *cad1* (*constitutively activated cell death 1*), shows activated plant immunity defense responses of HR (hypersensitive response) and SAR(systemic acquired resistance) (Plant Cell Physiol. 2005, 46: 902-912; Plant Biotechnol. 2011,28: 9-15). In this study, a potential F-box protein NIFC1, interacting with immune factor NSL2 is successfully detected by yeast two hybrid assay. NIFC1 combines with ASK1 through the N-terminal region to comprise SCF complex (PNAS. 2002, 99:11519-11524), which catalyzes as ubiquitin E3 ligase participated in UPS. Functional and physiological analysis of the NIFC1would shed a new light on UPS related plant immunity. Further characterizations of the NIFC1 which controls plant immunity will be reported.

PS01-022

Spatial and temporal cellular dynamics of the Arabidopsis flagellin receptor FLS2 reveal endosomal sorting depending on activation status

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Cell surface receptors mediate responses to environmental and developmental cues. The activity of surface receptors is location specific, dependent upon the highly dynamic membrane trafficking network and receptor-mediated endocytosis (RME). The spatio-temporal dynamics of RME are therefore critical to receptor function. The plant receptor kinase FLAGELLIN SENSING 2 (FLS2) is located at the plasma membrane and confers immunity against bacterial infection through perception of flagellin (flg22). Following flg22 elicitation, FLS2 is internalized into vesicles. To resolve FLS2 trafficking, we exploited quantitative confocal imaging for co-localisation studies and chemical interference. We developed EndomembraneQuantifier and EndomembranetCoLocQuantifier, two algorithms and software implementations for quantifying and identifying co-localised spot-like objects.In this study we defined the endocytic trafficking pathway of the A. thaliana FLS2 receptor in the absence of flg22 ligand and upon flg22-induced activation. FLS2 localises to bona-fide endosomes via two distinct endocytic trafficking routes depending on its activation status: FLS2 receptors constitutively recycle in a Brefeldin A (BFA)-sensitive manner while flg22-activated receptors traffic via ARA7/Rab F2b- and ARA6/Rab F1-positive endosomes, insensitive to BFA. In addition, flg22-induced FLS2 endosomal numbers increased by Concanamycin A (ConcA) treatment but reduced by Wortmannin (Wm) indicating that activated FLS2 receptors are targeted to late endosomal compartments. The RME inhibitors Endosidin 1 (ES1) and Tyrphostin A23 (TyrA23) did not block flg22-induced FLS2 endocytosis, These finding reveal that FLS2 employs an endocytic pathway distinct from other plant surface receptors and expose a dynamic pattern of subcellular trafficking for this immune receptor.

PS01-023

Tracking DIR1 movement and investigating the role of DIR1-like during Systemic Acquired Resistance in Arabidopsis

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Systemic Acquired Resistance (SAR) is initiated by some local infections leading to production of signals that move to and are perceived in distant leaves resulting in resistance to normally virulent pathogens. DIR1 participates in SAR long distance signaling as demonstrated by immunoblotting studies in which DIR1 is detected in SAR-induced wild type, but not *dir1-1* phloem sap-enriched exudates. Occasionally a DIR1-sized band is detected in *dir1-1* exudates suggesting that DIR1-like (At5g48490) may be involved in SAR. Additionally, recombinant protein studies demonstrate that DIR1 polyclonal antibodies recognize DIR1 and DIR1-like. Although, DIR1 and DIR1-like are 88% similar at the amino acid level, *dir1-1* was identified in a forward genetic screen and *dir1-1* is rarely SAR-competent. Use of a *dir1-1* line containing an estrogen-inducible DIR1-EGFP made it possible to visualize movement of DIR1 from a SAR-induced leaf down the petiole. DIR1 was also detected in distant leaf exudates, however the DIR1-GFP fusion protein was rarely observed in distant leaves of plants induced for SAR suggesting that GFP was cleaved from DIR1-GFP. Thus it was impossible to distinguish DIR1 from DIR1-like in distant tissues. To understand when and how DIR1-like contributes to SAR including the ability to distinguish DIR1 from DIR1-like, a *dir1-1 dir1-like* double mutant line is being subjected to stable and transient transformation with Agrobacterium expressing HA-tagged DIR1 and MYC-tagged DIR1-like. Moreover, experiments with the *dir1-1dir1-like* mutant may shed light on the variable SAR outcomes observed in a number of SAR labs (Cameron, Greenberg, Kachroo, Klessig, Shah, Zeier).

PS01-024

Expanding the paradigm of flagellin-triggered immunity: the importance of epitopes beyond flg22 and allelic diversity in both plant receptors and bacterial flagellins

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The flagellin (FliC) epitope flg22 is the archetypical plant immunity-triggering microbe-associated molecular pattern (MAMP) and is recognized in plants by FLS2, a pattern recognition receptor (PRR). We recently reported a second MAMP within flagellin that we termed flgII-28. This epitope corresponds to part of the region within flagellin that directly interacts with the vertebrate flagellin-receptor TLR5, thus revealing a surprising new parallel in plant and animal immunity. Different flgII-28 alleles exist in closely related pathogen strains and trigger immune responses of different strength in tomato suggesting that evasion of plant immunity through allelic variation in MAMPs is an important pathogen strategy. Reciprocally, different *Solanaceae* plants vary in their relative sensitivity to treatment with the different flgII-28 alleles, suggesting allelic variation in the corresponding flagellin receptors. Complementation of a *fliC*-deltion mutant of *Pseudomonas syringae* with *fliC* alleles identical in flg22, but different in flgII-28, results in bacteria with restored motility but significant differences in virulence on wild-type *Arabidopsis* but

not *fls2* mutants. We therefore conclude that MAMPs can vary in sequence without a negative effect on function (motility in the case of flagellin), and FLS2 seems to play a role in flgII-28 recognition. Some of our experiments even suggest that FLS2 is the flgII-28 receptor. However, contamination of flgII-28 with trace amounts of flg22 may have affected some of these experiments. We will further discuss the general risk of peptide contamination and experimental approaches to determine whether FLS2 or a yet unidentified PRR binds flgII-28.

PS01-025

Functional analysis of a Rice AAA-Type ATPase, an attentuator of programmed cell death

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Hypersensitive response (HR) is an effective mechanism used by plants to restrict the spread of pathogens. Rapid induction of cell death is a common phenomenon during HR. The potentially deleterious effects of the cell death and HR machinery demand a leak-proof attenuation system in the absence of pathogen infection. We identified a rice *Cdr2* gene which encodes an AAA-type ATPase that showed anti-apoptotic activity. The loss-of-function mutant cell death and resistance (*cdr2*) rice plants display a spontaneous cell death phenotype that resembles disease symptoms in the absence pathogen infection. This mutant lesion mimic mutant exhibits enhanced resistance to rice blast fungus infection. The *cdr2* mutation was localized by map-based cloning and DNA sequencing of candidate ORFs around the localized region and revealed that the cdr2 mutation is probably caused by a single-base (G to A) substitution, resulting in a Gly to Arg change in an ORF encoding an AAA-type ATPase. Overexpression of *Cdr2* gene in the cdr2 mutant complemented the lesion mimic phenotype and suppressed the cdr2 mutation-induced elevation defense-related genes. Furthermore, co-expression of *Cdr2* gene attentuated *Bax*-induced cell death in *Nicotiana benthamiana*. Taken together, Cdr2 may function as an attentuator in regulating cell death and defence response. To further analyse the function of Cdr2, using bioinformatics, we identified two potential interactors of Cdr2. Experiment is underway to characterise these interactors.

PS01-026

Arabidopsis transcriptional repressor ERF9 participates in resistance against necrotrophic fungi mediated by the DEAR1

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We characterized Arabidopsis *DEAR1* (*DREB AND EAR MOTIF PROTEIN 1*) and showed that overexpression of *DEAR1* (DEAR1ox) resulted in a dwarf phenotype and lesion-like cell death, accompanied by elevated expression of PR (Pathogenesis-Related) genes (Tsutsui et al. J. Plant Res. 2009). Here, we show that DEAR1ox has enhanced resistance to the necrotrophic fungus *Botrytis cinerea* (*B. cinerea*). This result implies that DEAR1 represses negative regulators of plant defense, for example transcriptional repressors which belong to the ERF (ETHYLENE RESPONSE FACTOR) family. Knockout mutants of ERF9 (*erf9*), the gene that was down-regulated in the DEAR1ox, showed transcriptional promotion of *PDF1.2* genes, which serve as positive markers for the ethylene/JA signaling pathway, and enhanced resistance to B. cinerea . Biochemical assays demonstrated that the ERF9 protein is capable of binding to the GCC box, a cis-element contained in the promoters of the *PDF1.2* gene and possessing trans-repression activity. Moreover, infection with *B. cinerea* resulted in the promotion of *PDF1.2* expression, coinciding with suppression of the *ERF9* gene under the control of the *DEAR1* gene. These results indicate that the transcriptional repressor ERF9 participates in plant defense against necrotic fungi mediated by the DEAR1 dependent ethylene/JA signaling pathway.

PS01-027

Conventional and unconventional functions of NLR immune receptors in *Arabidopsis*

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NLR (nucleotide-binding leucine-rich repeat) proteins represent the major class of intracellular innate immune receptors in plants which typically recognize specific pathogen effectors for defense responses. We previously showed that the ADR1 family of Arabidopsis NLR receptors differs from conventional NLR activation and functions. Specifically, the ADR1 proteins function as helper NB-LRRs to transduce signals downstream of specific NLR receptor activation during effector-triggered immunity, they are required for basal defense against virulent pathogens, and they regulate microbial associated molecular pattern-dependent salycilic acid accumulation induced by infection with a disarmed pathogen. Remarkably these functions do not require an intact P-loop motif (essential for nucleotide binding) for at least one ADR1 family member (ADR1-L2), suggesting that some NLRs with unconventional functions might act as scaffold for interactions with yet unknown immune partners.Although the nucleotide binding activity is not required for any of the described ADR1-L2 functions, a mutation in the conserved MHDV motif that is thought to favor an ATP-bound form of the NLR protein, leads to autoactivation phenotypes. An extensive epistasis analysis aimed to identify the genetic requirements of a P-loop-dependent autoactive allele of ADR1-L2 (D484V) will be described. Our data suggest a working model for the downstream signalling of activated NLR proteins, likely conserved among other immune receptors with conventional P-loop-dependent functions. In an effort to investigate the non-canonical functions of the ADR1 family, a biochemical approach will be described to elucidate the molecular mechanisms that regulates the ADR1 scaffold machinery and its components.

PS01-028

Short chitin oligomers from arbuscular mycorrhizal fungi trigger NFP-independent Ca^{2+} spiking in *Medicago truncatula* roots

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Arbuscular mycorrhizae (AM) are intracellular symbiotic associations between glomeromycetes and the roots of most land plants. It is currently thought that these widespread endosymbioses are initiated following reciprocal plant/fungal recognition in the rhizosphere, activating a plant signaling pathway partially shared with the rhizobial-legume symbiosis. A central element of this common symbiotic (SYM) pathway is the induction of nuclear Ca^{2+} oscillations with distinct signatures for AM fungi and rhizobia. We show here that characteristic Ca^{2+} spiking is induced in root organ cultures (ROCs) of the AM host *Medicago truncatula* by germinated spore exudates from glomeromycetes belonging to both Gigaspora and Glomus genera, but not from the pathogenic fungus Colletotrichum. Strikingly, a similar response can be triggered by short-chain chitin oligosaccharides (COs), with maximum activity

observed for chitin tetramers and pentamers. Although dependent on the common SYM pathway genes DMI1 and DMI2, CO-triggered spiking is not altered in a *M. truncatula nfp* mutant which is defective in the receptor for the rhizobial lipochito-oligosaccharide signals known as Nod factors. Furthermore, treatment with strigolactone, known to stimulate pre-infection AM hyphal ramification, induces a major increase in the concentration of CO4/5 in *Glomus* exudates. Structure/function experiments suggest that the addition of Nod factor-like decorations to the chitin backbone such as the sulphate moiety or the lipid chain significantly reduces spiking activity in *M. truncatula* ROCs. These findings indicate short-chain chitin oligomers as novel fungal signals perceived by a specific host receptor and are capable of activating an AM-dependent signaling pathway required for successful fungal colonization.

PS01-029

Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection

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Recognition of microbial patterns by host pattern recognition receptors is a key step in immune activation in multicellular eukaryotes. Peptidoglycans (PGNs) are major components of bacterial cell walls that possess immunity-stimulating activities in metazoans and plants. Here we show that PGN sensing and immunity to bacterial infection in *Arabidopsis thaliana* requires three lysin-motif (LysM) domain proteins. LYM1 and LYM3 are plasma membrane proteins that physically interact with PGNs and mediate *Arabidopsis* sensitivity to structurally different PGNs from gram-negative and gram-positive bacteria. *lym1* and *lym3* mutants lack PGN-induced changes in transcriptome activity patterns, but respond to fungus-derived chitin, a pattern structurally related to PGNs, in a wild-type manner. Notably, *lym1*, *lym3*, and *lym3 lym1* mutant genotypes exhibit supersusceptibility to infection with virulent *Pseudomonas syringae* pathovar *tomato* DC3000. Defects in basal immunity in *lym3 lym1* double mutants resemble those observed in *lym1* and *lym3* single mutants, suggesting that both proteins are part of the same recognition system. We further show that deletion of CERK1, a LysM receptor kinase that had previously been implicated in chitin perception and immunity to fungal infection in *Arabidopsis*, phenocopies defects observed in *lym1* and *lym3* mutants, such as peptidoglycan insensitivity and enhanced susceptibility to bacterial infection. Altogether, our findings suggest that plants share with metazoans the ability to recognize bacterial PGNs. In further studies we investigate the molecular mechanism of the PGN LYM1/LYM3 interaction. To deepen the understanding of PGN as an immunostimulatory ligand we are also determining the minimal PGN epitope needed for PGN perception.

PS01-030

Toxin-mediated release of DAMPs - A novel trigger of plant innate immunity

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The activation of innate defense mechanisms of plants against microbial infection is mainly based on two branches: the PRR-mediated recognition of PAMPs (pathogen-associated molecular patterns), called PAMP-triggered immunity and the recognition of microbial effectors by receptors encoded by R-genes, called effector- triggered immunity. Besides these two mechanisms of pathogen perception, plants can also sense endogenous patterns, representing stress-associated molecules (damage-associated molecular patterns, DAMPs), which induce innate immunity. Such endogenous elicitors so far comprise cell wall fragments, cutin monomers and peptides like systemin and AtPEP1.Well-known triggers of plant immune responses are necrosis and ethylene-inducing peptide 1-like proteins (NLPs). NLPs are virulence-promoting toxins found in phytopathogenic bacteria, oomycetes and fungi. By disrupting the plasma membrane of dicotyledonous plants, NLPs are inducing cell death and thus contribute to the virulence of necrotrophic and hemibiotrophic plant pathogens. The mechanism of membrane disruption might be similar to that of structurally related pore-forming toxins from marine invertebrates, but remains to be elucidated.How NLPs induce immunity-associated responses in dicotyledonous plants is still unknown. Studies with active and inactive mutant versions of NLPs showed, that not the NLP molecule itself is recognized, but its membrane disrupting activity. Thus, it is very likely that the activity of NLPs induces the production of breakdown products or the release of intracellular molecules that are sensed as DAMPs. The identification of those plant-derived DAMPs and their corresponding receptors will help to elucidate this novel form of plant innate immunity.

PS01-031

A simple model system for detecting metabolic changes in symbiotic *Nostoc punctiforme*

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Nostoc punctiforme is a filamentous, nitrogen fixing cyanobacterium. It is capable of forming symbiotic relationships with diverse plants and fungi. It can grow heterotrophically without light. In the absence of combined nitrogen heterocysts will form. Heterocysts are cells capable of fixing nitrogen. Plants utilize *N. punctiforme's* nitrogen fixing ability, allowing it to fix nitrogen inside the root tissue of plants. This symbiotic relationship functionally resembles nitrogen fixation by rhizobia. Since light cannot penetrate the root system, *N. punctiforme* must rely on organic carbon from the host for survival. The cultivation of *N. punctiforme* in the dark is a simple model to analyze its metabolism in the stage of symbiosis. The light state represents the free living form, whilst the dark state mimics the symbiotic form. We have cultured strains of *N. punctiforme* with and without glucose, fructose and sucrose in the presence and absence of combined nitrogen, and in light and dark. In both light and dark, in the absence of combined nitrogen with either of the three sugars, samples showed faster growth and higher concentration of heterocysts than non sugar samples. We have initiated microarray experiments in order to compare global gene expression patterns in light and dark states. This will lead to a better understanding of the metabolic transitions of *N. punctiforme* cells necessary to establish symbiosis.

PS01-032

Characterization of rice chitin elicitor receptor complex

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Plants have the ability to recognize microbe/pathogen- associated molecular patterns (MAMPs/ PAMPs) and initiate various defense responses. Chitin is a major MAMP for various fungi and its fragments, chitin oligosaccharides, trigger defense responses in a wide range of plant species. Recently, we identified two types of chitin receptors, CEBiP (1) and OsCERK1 (2), which play an important role for chitin elicitor signaling in rice. We also

showed that both molecules formed a receptor complex in the presence of chitin oligosaccharide (2). OsCERK1 is a receptor-like kinase with single transmembrane region, whereas CEBiP is a GPI-anchored protein and at least parlty present in the lipid raft of the plasma membrane. To investigate how these structurally different molecules can form the complex, we prepared the lipid raft fraction from plasma membrane of rice cells treated with and without chitin elicitor, and analyzed the behavior of these receptors. (1) Kaku *et al.*, *PNAS*, 103, 11086 (2006); (2) Shimizu *et al.*, *Plant J.*, 64, 204 (2010).

PS01-033

Understanding Cf-9 signal activation through molecular genetic dissection of an autoactive mutant, M205

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The tomato *Cf-9* gene confers resistance to races of the fungal leaf mould pathogen, *Cladosporium fulvum*, that express the *Avr9* avirulence gene. *Cf-9* encodes a receptor-like protein containing a large plasma membrane-bound extracytosolic leucine-rich repeat (LRR) domain and a short cytosolic tail. An autoactive mutant of Cf-9 designated M205 has been described previously. This mutant consists of an in-frame fusion encoding the N-terminus of an upstream paralogue, Cf-9A, and the C-terminus of Cf-9. Transient expression of M205 in tobacco causes hypersensitive response (HR) in the absence of Avr9. This study employed domain swapping and site-directed mutagenesis to identify regions and amino acid residues required for M205 autoactivity by scoring HR upon transient expression of the domain swaps/mutant constructs in tobacco. Domain swapping between Cf-9 and Cf-9A revealed that substitution of Cf-9A sequence into LRRs13-17 of Cf-9 is sufficient to induce autoactivity. We postulate that LRRs13-17 are involved in signalling repression necessary to keep Cf-9 inactive in the absence of Avr9 and that the Cf-9A substitution disrupted repression and allowed autoactivity. We are currently investigating the contribution of polymorphic residues in LRRs13-17 to autoactivity by site-directed mutagenesis. Interestingly, residues involved in Avr9 recognition overlap the polymorphic residues, suggesting the overlapping positions may participate in both Avr9 recognition and signalling repression. By interchanging Cf-9A and Cf-9 residues at these positions to look at the loss of autoactivity in M205 or gain of autoactivity in Cf-9, key residues involved in signalling repression have been identified.

PS01-034

The N-terminal MAPK-docking site in tomato MAPK kinase SlMKK2 is required for interaction with a downstream MAPK to trigger programmed cell death associated with plant immunity

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A mitogen-activated protein kinase (MAPK) cascade is one of the key signal transduction pathways regulating immunity-associated programmed cell death (PCD) in plants. Previously SlMAPKKKα, a tomato MAPK kinase kinase and SlMKK2, a MAPK kinase were shown to be required for elicitation of PCD mediated by the Pto disease resistance protein upon recognition of the effector proteins AvrPto or AvrPtoB from *Pseudomonas syringae* pv. *tomato*. A 14-3-3 protein, TFT7, was found to interact with SlMKK2 at its N-terminus in a region overlapping the MAPK-docking site (or D-site). Here, we examine the role of the D-site of SlMKK2 in PCD elicitation. *In vivo* assays revealed that SlMKK2, but not TFT7, interacted with the MAPK SlMPK3 independent of PCD elicitation. The N-terminal D-site of SlMKK2 was required for both interaction with SlMPK3 and $SlMKK2^{DD}$-mediated PCD in plants. In particular, two conserved leucines in the D-site of SlMKK2 were required for interaction with SlMPK3. Consistent with this, co-expression of SlMPK3 with $SlMKK2^{DD}$ enhanced PCD mediated by $SlMKK2^{DD}$ and PCD mediated by a $SlMKK2^{DD}$ derivative, in which the two conserved leucines were substituted with alanine. These results demonstrate that the D-site of SlMKK2 plays a critical role in regulation of signal transfer to the downstream component SlMPK3 by regulating their physical interaction.

PS01-035

Identification and molecular characterization of novel PAMPs from the gram-negative bacterium *Ralstonia solanacearum* in *Arabidopsis thaliana*

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Plant innate immunity is activated either upon perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) or upon resistance (R) protein-mediated recognition of pathogen race-specific effector molecules. PAMP-triggered immunity (PTI) constitutes the primary plant immune response that has evolved to recognize invariant structures of microbial surfaces. Although several cell surface components of bacteria, fungi or oomycetes have been shown to act as PAMPs that trigger immune responses in various plant species, the enormous non-self recognition capacities of plants are not at all explored. To find new proteinaceous PAMPs we used crude protein preparations of the phytopathogenic gram-negative bacterium *Ralstonia solanacearum*. We found a proteinase-sensitive and heat stable PAMP activity in these crude extracts which triggers immune responses in the model plant *Arabidopsis thaliana* such as ethylene production, PR1 induction and medium alkalinization. We further fractionated the bacterial proteins by different chromatography strategies such as ion exchange chromatography. In further purification steps we will now aim at isolating the protein that is responsible for stimulating immunity in Arabidopsis.

PS01-036

Cysteine-rich receptor like kinase family members are differentially activated by powdery mildew infection in susceptible and mlo-resistant barley

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The receptor-like protein kinases constitute a large and diverse group of proteins controlling numerous plant physiological processes, including development, hormone perception and stress responses. Transmembrane-anchored cysteine rich receptor-like protein kinases (CRKs) represent a prominent sub-family of RLKs in plants. In barley we have identified 39 members of the CRK family that are transcriptionally active in response to various biotic and abiotic stresses. Most of the CRKs encode putative proteins with an N terminal receptor containing two characteristic duf26 domains followed by a transmembrane domain. The C terminal part comprises of a highly conserved protein kinase domain. The CRK genes consist of seven exons and cluster on chromosome 2HS and 5HL, but are found on all chromosomes. Expression profiling of the CRK family members revealed a transient increased accumulation of eight CRalK transcripts following inoculation of susceptible barley with the biotrophic fungus *Blumeria graminis* f.sp. *hordei* (Bgh), in contrast these transcripts was not induced in mlo-resistant barley. Silencing of one of these, *HvCRK1*, by transient gene silencing led to an enhanced penetration resistance

to Bgh, but did not affect R-gene mediated resistance. Interestingly, three other CRKs were found to accumulate specifically in mlo-resistant barley and not in susceptible barley after Bgh attack. Our results suggest that CRKs in barley are involved in separating and mediating signals for susceptibility and basal resistance responses. A simple comparison of known promoter elements did not reveal any obvious differences between the CRKs activated in susceptible and mlo-resistant barley.

PS01-037

Functional analysis of BAK1-interacting protein 89 in plant innate immunity

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The LRR-RLK BAK1 interacts with the brassinosteroid receptor BRI1, the flagellin receptor FLS2 and the EF-Tu receptor EFR to control brassinosteroid and MAMP signaling. Furthermore, BAK1-deficient plants develop spreading necrosis after *Alternaria brassicicola* infection. This phenomenon is independent of the brassinolid pathway, suggesting a role of BAK1 in pathogen induced cell death control. In order to find new BAK1-interacting proteins that might be involved in the regulation of plant cell death control, co-immunoprecipitations of *in vivo* BAK1 complexes followed by MS analyses of the interaction partners have been performed. We identified a new RLK, named BIP89 for BAK1-interacting protein 89. The expression level of BIP89 increases upon biotrophic pathogen and MAMP treatment pointing to a role in pathogen defense signaling. Two *Arabidopsis* mutant lines as well as artificial microRNA lines have been used in different assays to decipher the function of BIP89. Interestingly, BIP89-deficient plants exhibit higher sensitivity to the MAMPs flg22 and elf18 than the respective wild-type plants. Bacterial infection experiments show that mutants limit the growth of *Pseudomonas syringae* pv. *tomato* DC3000 compared to the wildtype control. On the other hand, these mutants show stronger symptom development than wildtype and *bak1-4* mutant plants upon infection with the necrotrophic fungus *Alternaria brassicicola*. Taken together, these results suggest a functional link of this protein to BAK1 regulated processes and provide another layer of complexity in BAK1 receptor complex formation. Here, we will present current data on the function of BIP89 in modulation of BAK1 regulated processes.

PS01-038

Ethylene responsive AP2/ERF transcription factor MACD1 participates in phytotoxin-triggered programmed cell death

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Programmed cell death (PCD), known as hypersensitive response cell death, has an important role in plant defense response. The signaling pathway of PCD remains unclear. To analysis plant PCD, we employed AAL-toxin and *Nicotiana umbratica*. AAL-toxin is a pathogenicity factor of necrotrophic pathogen *Alternaria alternata* f. sp. *lycopersici* and triggers PCD in AAL-toxin-sensitive *N. umbratica*. Our recent work demonstrated that MAPK cascades and ethylene (ET) signaling play pivotal roles in AAL-toxin-triggered cell death (ACD). We also showed that Modulator of AAL-toxin Cell Death 1 (MACD1), which is an AP2/ERF transcription factor, participates in ACD. The necrotic lesion of ACD emerged more rapidly in MACD1 overexpression (OE) plants compared with control plants. To further investigate roles of ET signaling in PCD, we employed *Arabidopsis thaliana* and structural analog of AAL-toxin, fumonisin B1 (FB1). FB1-tirggered cell death (FCD) was compromised in ET signal mutants and *Atmacd1* mutants. AtMACD1 is the transcriptional activator and AtMACD1 OE plants also displayed earlier FCD induction than Col-0 plants, suggesting that AtMACD1 positively regulates the factors affecting cell death development. Furthermore, *loh2* mutants showed sensitivity to AAL-toxin and *loh2/atmacd1* mutants compromised ACD, indicating that AtMACD1 also participates in ACD in *A. thaliana*. To investigate the PCD-associated genes regulated by AtMACD1, we identified up-regulated genes in AtMACD1 OE plants by microarray analysis. We compared our microarray data with the database of up-regulated genes by AAL-toxin treatment in *loh2* mutant and, isolated the genes under control of AtMACD1 in ACD.

PS01-039

A moss MAP kinase required for PAMP triggered immunity and defence against necrotrophic fungi

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Plant mitogen-activated protein kinase (MPK) cascades convert extracellular stimuli into cellular responses, and activation of MPK signaling is required for the induction of innate immunity in higher plants. Perception of pathogen-associated microbial patterns (PAMPs) by trans-membrane pattern recognition receptors initiate MPK-dependent PAMP-triggered immunity (PTI). In the model dicot Arabidopsis, 3 of some 20 MPKs (MPK3/4/6) are implicated in PTI although the importance of these single MPKs is still under debate. The genome of the haploid moss Physcomitrella only encodes 8 MPKs, and we provide here a primary example of a Physcomitrella MPK that is essential for PTI. More specifically, we demonstrate that knock-out of Physcomitrella PpMPK4 (PpdeltaMPK4) renders the moss more susceptible to the necrotrophic fungi Botrytus cinera and Alternaria brassisicola. Concordingly, several defence-related transcripts fail to accumulate in the PpdeltaMPK4 mutant upon infection or treatment with the fungal elicitor chitin. While Arabidopsis MPK3/4/6 are all activated by differrent abiotic stresses, we did not detect activation of PpMPK4 or any other Physcomitrella MPK by abiotic stresses. Signal transduction via PpMPK4 may therefore be specific to PAMP perception. These results demonstrate that Physcomitrella is an appropriate model to understand the contribution of single MPKs in responses to stimuli including PTI.

PS01-040

Characterization of putative *Arabidopsis thaliana* MAP-kinase substrates related to defense responses

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Mitogen-activated protein kinase (MAPK) cascades mediate diverse cellular signal transduction processes. These include growth and developmental, as well as stress-related responses important for the adaptation to adverse environmental conditions. In our lab, we conducted yeast-2-hybrid and protein array screens to identify interacting proteins and substrates of the MAPKs, MPK3 and 6. Among the putative substrates, potential signaling components like a casein kinase-like protein or ACC-synthase 6, and transcriptional regulators like SNF2 (SWI/SNF-type ATPase), MYB88 or a Plant Homeo Domain (PHD)-containing protein were

identified. Selected candidates were expressed in *E. coli* and the purified recombinant proteins were used for *in vitro* kinase assays to confirm phosphorylation by the MAPKs. Phosphopeptide enrichment and mass spectrometry was employed to identify the phosphorylation sites. In parallel, we also developed an efficient site-directed mutagenesis strategy to generate individual and higher order phospho-site mutants of MAPK substrates. Transient expression in Arabidopsis protoplasts was then used for in vivo characterization, aiming at unravelling the role of phosphorylation of MAPK substrates in pathogen-associated molecular-pattern-triggered signaling.

PS01-041

Identification and characterization of the novel fungal MAMP SsE1 and its RLP-based recognition complex in *Arabidopsis thaliana*

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Effective plant defence strategies rely on the perception of non-self determinants, so-called microbe-associated molecular patterns (MAMPs), by transmembrane pattern recognition receptors (PRRs) and on the resulting induction of corresponding immune responses. We semi-purified a novel proteinous elicitor called SsE1 (*Sclerotinia sclerotiorum* Elicitor1) from the necrotrophic fungal pathogen *Sclerotinia sclerotiorum* which promotes typical MAMP-induced defence responses in the model plant *Arabidopsis thaliana*. SsE1-triggered defence responses require the presence of the leucine-rich repeat receptor-like protein SER1 (*Sclerotinia* Elicitor Receptor 1) and the leucine-rich repeat receptor-like kinase BAK1. The general objectives of this project will be to elucidate the nature of SsE1 as well as to decipher the molecular mechanisms underlying the perception of SsE1 and the subsequent signal transduction via SER1-BAK1. Our work will deepen our mechanistic understanding on how plants adapt to a detrimental biotic environment.

PS01-042

DAMP signalling in plant innate immunity

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The activation of plant innate immunity involves specific detection of pathogen associated molecular patterns (PAMPs) by corresponding host encoded pattern recognition receptors (PRRs). Perception of PAMPs will subsequently trigger signalling cascades leading to activation of plant defences. In addition to recognition of PAMPs, plants have the ability to recognize modified-self, including damage-associated molecular patterns (DAMPs). A major category of DAMPs are plant cell-wall fragments released by the action of degradative enzymes secreted by the pathogen during the infection process. Release and recognition of such fragments has also been shown to trigger innate immunity responses and result in enhanced disease resistance. Our objective is to elucidate the molecular mechanisms involved in DAMP signalling in *Arabidopsis thaliana* using oligogalacturonides (OGs), released during the infection by the soft-rot pathogen *Pectobacterium carotovora*, as the model DAMPs. We hypothesize that our genetic screens for OG-insensitive mutants will allow identification and characterization of central components in the signalling pathways triggered by plant recognition of OGs. From the screen several lines with increased susceptibility to pathogens, as well as those with altered development, have been identified - highlighting the connection between development and innate immunity. The preliminary results would seem to indicate that we have been able to isolate potential DAMP signalling components.

PS01-043

***In planta* identification and functional analysis of EFR complex components**

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Plants recognize conserved microbial molecules, called pathogen-associated molecular patterns (PAMPs), by pattern-recognition receptors (PRRs) and induce defense responses leading to PAMP-triggered immunity (PTI). The leucine-rich repeat receptor kinases, EFR and FLS2 are the PRRs for the bacterial PAMPs flagellin (or the derived peptide flg22) and EF-Tu (or the derived peptide elf18), respectively. To understand the molecular mechanism underlying activation of PTI from PRRs at the plasma membrane to downstream signaling events, we sought to identify proteins that associate with EFR in Arabidopsis. For this purpose, we immunoprecipitated physiological levels of EFR-GFP using magnetic beads and identified EFR-associated proteins by mass-spectrometry analyses. This approach allowed high specificity and the recovery of large amount of previously known EFR interactors, but also enabled the discovery of novel EFR complex components. Novel candidate complex components include (1) proteins whose gene expressions are highly activated after PAMP treatment, (2) proteins that are known to be highly phosphorylated after PAMP treatment, (3) proteins known to be associated with ETI components. The possible roles of these candidates in PTI will be discussed.

PS01-044

Phosphoproteomics of MAMP-triggered immunity

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Proteomics is one of the best available tools for studying posttranslational modifications (PTMs). Therefore, it is well suited for dissecting signaling pathways which often utilize PTMs as a means of transmitting signals. Among the several PTMs described thus far, phosphorylation is the most extensively studied and has been shown to play an important role in almost all basic cellular processes in plants. Accordingly, we have been developing shotgun phosphoproteomics platform to dissect out poorly characterized signal transduction systems by monitoring phosphorylation dynamics. We have so far reported that we enabled to routinely identify thousands of phosphoproteins from plant materials (1, 2). Furthermore, we have established methods for quantitative analyses. Using the established platform, we examined phosphoproteome changes in Arabidopsis upon MAMP (microbe-associated molecular pattern) treatment to identify novel players involved in MAMP-triggered immunity. As a result, we identified hundreds of proteins whose phosphorylation status significantly changed in response to MAMP treatments. The identified proteins included well known MAMP signaling regulators. However, most of the proteins were not reported to take part in MAMP signaling. To verify involvement of the identified proteins in MAMP-triggered responses, we have been isolating T-DNA insertion lines for these proteins and characterizing flg22-induced ROS (reactive oxygen species) production in the isolated mutants. We have so far identified several negative and positive regulators of MAMP-induced ROS production, and are expecting to isolate more MAMP-signaling regulatory genes with continued screening.
(1) N. Sugiyama et al., Mol Syst Biol. 4: 193 (2008); (2) H. Nakagami et al., Plant Physiol. 153(3): 1161-74 (2010).

PS01-045

The ubiquitin ligases PUB22, PUB23 and PUB24 regulate PAMP-triggered responses by targeting a component of the exocytic pathway

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The closely related plant u-box type E3 ubiquitin ligases (PUBs) PUB22, PUB23 and PUB24 are involved in the regulation of innate immunity signalling. Perception of pathogen-associated molecular patterns (PAMPs) is mediated by plasma membrane localized receptors and activation results in a plethora of responses. The *pub22/pub23/pub24* triple mutant displays an increased and prolonged activation of early signalling events after perception of various PAMP. This suggests that the PUB triplet modulates a common cellular process required for the down-regulation of signalling mediated by various pattern recognition receptors (PRRs). To isolate ligase targets responsible for this phenotype, we performed a yeast two-hybrid screen with PUB22, from which we identified several candidate interactors. These included a subunit of the exocyst, an octameric complex which mediates early steps of exocytosis. We show that the PUB triplet interacts with this subunit *in vivo* and that they mediate its turn-over. Furthermore, degradation of the ligase target is regulated by the stability of the ligase itself, which displays auto-ubiquitination activity. Mutant analysis confirmed that the exocyst subunit is required for early PAMP-triggered signalling, as evidenced by decreased PAMP-triggered responses and decreased resistance against different plant pathogens. In summary, we will present data that supports a mechanism in which PUB22, PUB23 and PUB24 contribute to the regulation of PAMP-triggered responses by targeting components of exocytic machinery.

PS01-046

Artificial evolution of the NB-LRR, Rx, to enhance activation sensitivity in a broad recognition background

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Plant Resistance (R) genes provide protection against a diverse range of pathogens, from nematodes to viruses, with the vast majority encoding proteins from the nucleotide binding leucine-rich repeat (NB-LRR) class. The C-terminal LRR region is thought to provide recognition specificity, while the N-terminal NB containing region activates downstream signalling leading to a defense response. Previous results from our lab demonstrated that, in the R gene (Rx), the LRR region can be artificially evolved to recognise viruses undetected by the wild type Rx protein. However, some of these broad recognition versions suffer from a reduced activation response, with deleterious consequences on plant fitness. During my doctoral research, I performed random mutagenesis on the N-terminal activation domains of a broad recognition version of Rx, and screened approximately 1500 clones for increased activation characteristics. I isolated four Rx mutants that show increased defense response without constitutively activating the protein, while retaining the broad recognition phenotype. Through homology modelling, we also revealed that these mutations concentrate in a feature of Rx that is conserved across all known NB-LRRs proteins. This strategy of targeted evolution, where recognition and activation characteristics are sequentially modified, could potentially be employed to improve disease resistance in crops.

PS01-047

Characterization of calcium signalling mutants in *Arabidopsis thaliana* innate immunity

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During attempted infection of plants, pathogens are betrayed by conserved "Microbe-Associated Molecular Patterns" (MAMPs) that are recognized by specific host receptors and initiate intracellular signalling cascades leading to MAMP-triggered immunity. Endogenous "Damage-Associated Molecular Patterns" (DAMPs) similarly elicit receptor-mediated defences. Rapid elevations in the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) are a core component of MAMP and DAMP signal transduction and are crucial for establishment of downstream responses, such as reactive oxygen species (ROS) accumulation, activation of protein kinases, and induction of defence gene expression. The MAMPs flagellin (flg22), elongation factor Tu (elf18) and chitin, as well as the DAMP AtPep1 provoke generally similar prolonged $[Ca^{2+}]_{cyt}$ elevations in *Arabidopsis thaliana* but with distinct lag times and amplitudes. Mutant analysis revealed a feedback impact of the Ca^{2+}-dependent ROS accumulation on the $[Ca^{2+}]_{cyt}$ elevation. Despite the pivotal role of Ca^{2+} as second messenger in MAMP signalling, only a few participating Ca^{2+} channels and transporters are known. Using chemically mutagenised Arabidosis seedlings expressing the Ca^{2+}-reporter aequorin, we isolated mutants with *changed calcium elevation* (*cce*) in response to flg22. These comprise novel alleles of the flagellin receptor FLS2 and the receptor-associated kinase BAK1, as well as other cce mutants with partially reduced or enhanced $[Ca^{2+}]$ elevations in response to several MAMPs and DAMPs. Thus, these *CCE* genes encode components shared by different MAMP/DAMP signalling pathways and will be useful to unravel early signalling events in plant-microbe interactions. Currently, we are identifying the *CCE* genes by mapping and genome sequencing and characterising their role in innate immunity.

PS01-048

Receptors-like kinases go endosomal: a family picture of dynamic PRR subcellular localization in a ligand-specific manner

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Plants perceive conserved pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) through plasma membrane-localized receptors referred to as pattern recognition receptors (PRRs). Signalling events triggered by PRRs result in increased plant defences against potential invasive pathogens, a phenomenon known as PAMP-Triggered Immunity (PTI). Several PRRs have been described and among them, FLAGELLIN SENSING 2 (FLS2), from Arabidopsis thaliana, is one of the best characterized and confers resistance to bacterial infection through the recognition of bacterial flagellin (flg22). After perception of flg22, FLS2 relocates from the plasma membrane and is internalized within minutes. Using transient expression in *Nicotiana benthamiana* coupled to live cell confocal imaging, here we demonstrate that

four different fluorescent-tagged PRRs including AtFLS2 are internalized in a ligand-dependent and -specific manner. Vesicles labelled with the PRRs after ligand treatments co-localize with RabF2B, a reported marker of late endosomes but not with a marker of the secretory pathway. This response depends on the function of NbSERK3a and NbSERK3b - two undistinguishable homologues of Arabidopsis thaliana BAK1/SERK3 known to form a complex with several receptor-like kinases including FLS2 - as shown by Virus Induced Gene Silencing (VIGS) experiments. These results are consistent with knowledge from AtFLS2 trafficking in Arabidopsis and show that PAMP/DAMP-induced endocytosis of PRRs is conserved in N. benthamiana. It also demonstrates that ligand-induced internalization is a mechanism present among different PRRs, supporting the idea that this plays a critical function for plant defence responses.

PS01-049

Evasion of host immune recognition of flagellin in plant and human cells by bacterial pathogens

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The innate immune system in plants and animals is equipped with receptors that recognize pathogen associated molecular patterns (PAMPs) that upon PAMP recognition trigger an arsenal of downstream defense responses. Successful pathogens prevent the activation of defenses by (i) suppression of host defense responses via the release of effector proteins, and/or (ii) evasion of recognition by its host. Here, we show that the mammalian and plant pathogens *Pseudomonas aeruginosa* and *P. syringae*, respectively, display a strategy that prevents host immune detection of the PAMP flagellin by the TLR5 receptor in human cells and the FLS2 receptor in plant cells. In our search for agonists of these flagellin receptors we identified the alkaline protease AprA as signaling inhibitor (Bardoel *et al*. 2011, *PLoS Pathog*. 7: e1002206). AprA specifically cleaves monomeric flagellin molecules, while polymeric flagellin resists degradation. *P. aeruginosa aprA* mutants induced a 100-fold enhanced activation of TLR5 signaling in mammalian cells, because they fail to degrade excess monomeric flagellin in their environment. In Arabidopsis, AprA also prevents flagellin mediated responses, such as growth inhibition and callose deposition. Furthermore, faster stomatal closure after treatment with *P. aeruginosa aprA* mutants compared to *P. aeruginosa* wild-type bacteria was observed. In addition, *P. syringae aprA* mutants are less virulent and induce a stronger defense gene expression in their host. Thus, the bacterial protease AprA degrades excess flagellin monomers and in this way enables pathogenic bacteria to escape recognition by the innate immune systems of their host.

PS01-050

The PAMP-triggered immunity response is involved in the plant defense response to aphid attack and is suppressed by an aphid effector

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Aphids are insects which feed on phloem sap using their stylets. This can lead to significant losses of crop yield, both through the drain to plant resources and the vectoring of plant viruses. Due to these negative effects, it would be expected that plants have developed defenses against them. In plant-pathogen interactions, basal plant defense involving pathogen associated molecular pattern (PAMP) triggered immunity (PTI) effectively fends off the majority of plant pathogens. We have found that aphids can evoke typical PTI responses such as ROS bursts and callose deposition. In order for successful colonization by a pathogen, the PTI pathway is targeted using effectors, which manipulate plant processes to enhance susceptibility to the invading pathogen. Successful colonization of a host by an aphid is also thought to involve effectors which are most likely salivary gland proteins that are introduced into the plant during aphid feeding. Previously a salivary gland protein from the aphid Myzus persicae, Mp10, was identified which suppresses the Reactive Oxygen Species (ROS) burst elicited by the PAMP flg22 (Bos, Prince et al., 2010. PLoS Genetics 6(11): e1001216). Further investigation of Mp10 function revealed that it also suppresses the calcium burst that precedes the flg22 ROS burst, as well as the ROS burst elicited by crude aphid extract. In conclusion, our results so far indicate that PTI plays a role in plant defense response to aphid attack and is suppressed by an aphid effector.

PS01-051

Screening proteins with "VQ"motif: The quest for MAPK substrates involved in plant immunity

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Plants recognize potential pathogens by receptor-mediated detection of conserved microbial structures, so-called pathogen-associated molecular patterns (PAMPs). This initiates signalling pathways, including mitogen activated protein kinase (MAPK/MPK) cascades, which transduce such extracellular PAMP signals into the appropriate defence responses. The same MAPK component often participates in diverse pathways and it is thought that the signal fidelity may be provided by specific incorporation into different protein complexes and/or phosphorylation of distinct MAPK substrates. MAPK substrates identified to-date include the MPK4 substrate 1 (MKS1) that regulates defence gene expression *via* interaction with the WRKY transcription factor WRKY33. MKS1 is a member of the "VQ" motif containing protein (VQP) family. In a yeast two hybrid screen against an *Arabidopsis* cDNA library, we identified three additional VQPs that interact with the PAMP-activated MPKs, MPK3 and MPK6. We will present our studies involving all Arabidopsis VQPs, analysed by *in vitro* kinase and yeast two hybrid assays. Here, we identified further VQPs that interacted with and were phosphorylated by MPK3 and MPK6, and, therefore, are possibly involved in signalling PAMP-induced defence responses.

PS01-052

JAZ protein is a critical component of stomatal immunity

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Stomata are the natural openings formed by a pair of guard cells present on the leaf surface that close in response to live bacteria and bacterial motifs as a part of innate immune responses. Stomatal closure is effective in restricting bacterial penetration into leaf tissues; however phytopathogenic bacteria such *Pseudomonas syringae* pv. *tomato* (Pst) strain DC3000 produce the virulence factor coronatine that counteracts stomatal immunity. Recent research allowed for the identification of COI1 (the F-box subunit of the SCF^{COI1} E3 ubiquitin ligase) and JAZ [a negative regulator of the jasmonic acid (JA) pathway] as co-receptors of coronatine establishing that JA signaling contributes to coronatine-dependent disease progression. Using a combination of approaches including gene expression analysis, yeast-two-hybrid system, ectopic expression of truncated proteins, gene knockouts, and stomatal assays we discovered that JAZ-meditated repression of JA contributes to stomatal immunity in Arabidopsis. Specifically we

have determined that *JAZ* genes are induced by coronatine in guard cells and structural-functional relationship analyses revealed the domains of JAZ proteins necessary for their function. Finally, we assessed the role of naturally occurring splice variants of JAZ in Arabidopsis stomatal defense against Pst DC3000. The phenotype of these plants and the biological significance of these findings will be further discussed.

PS01-053

Evaluation of PAMP-triggered immunity for developing durable disease control in barley and wheat.

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Plants detect pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), which activate basal and non-host defence responses. PAMPs are important conserved molecules, whose loss or mutation cannot be easily selected for, and thus PRRs could potentially offer broad-spectrum durable resistance to pathogens. The best-studied bacterial PAMPs are flagellin and elongation factor-Tu (EF-Tu), recognised by the Arabidopsis leucine-rich repeat receptor-like kinases (LRR-RLKs) FLS2 and EFR. Chitin, a major constituent of fungal cell walls, is the best-studied fungal PAMP, and is recognized by the LysM domain-containing receptor-like protein (RLP) CEBiP (in rice and barley) and the LysM-RLK CERK1 (in rice and *Arabidopsis*). PAMP-triggered immunity (PTI) may contribute towards quantitative (or partial) disease resistance (QDR), an attractive target for crop breeding. Our objectives were to develop methods for studying PTI in wheat and barley, and to define how PTI contributes to QDR as a basis for crop improvement. In barley, we found evidence for induction of defence genes and resistance to pathogens such as *Pseudomonas syringae* pv. *syringae* after treatment with PAMPs. Given the role of CEBiP in chitin perception, we are studying how CEBiP expression levels contribute to PTI and QDR. Based on recent evidence that PRRs can be transferred across plant families to confer broad-spectrum resistance, we have also transformed *AtEFR* and *AtCERK1* into wheat and barley to test whether their overexpression confers elevated PAMP responses and increased disease resistance. Our research will enable us to evaluate how PTI can be exploited in agriculture to develop broad-spectrum quantitative disease resistance.

PS01-054

The host cell actin cytoskeleton is altered in plants infected with *Pseudomonas syringae*

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Changes to the plant actin cytoskeleton have been observed in responses to infection by fungal and oomycete pathogens. However, similar responses have not been thoroughly described, nor investigated, in the case of phytopathogenic bacteria. Tian *et al*. (2009) first reported evidence for a relationship between the actin cytoskeleton and defense against bacterial infection. In this study, we investigated quantitatively actin organization in *Arabidopsis* plants infected with *Pseudomonas syringae* pv. tomato DC3000 using laser scanning confocal microscopy. We have established a protocol using 10 days-old *Arabidopsis* seedlings for infection and confocal microscopy analysis. Transgenic Col-0 seedlings expressing the actin marker, ABD2fGFP, were dip-inoculated with virulent or avirulent *P.syringae*. The disease phenotype of seedlings expressing GFPfABD2 showed the same trend as Col-0 mature plants, showing that seedlings can be used to study host cell-bacteria associations. We observed a significant increase in actin filament bundling and a significant decrease in overall filament density in seedlings inoculated with *Pst* DC3000 EV compared with mock-treated controls at 24-28 hours after inoculation. In contrast, actin skewness and density in seedlings inoculated with DC3000 D28E and HrpH- did not show any significant differences when compared with mock, suggesting involvement of the Type III Secretion System (T3SS) of actin dynamics. In summary, this work provides further evidence that *P. syringae* engages the host actin cytoskeleton during infection, and moreover, supports our hypothesis that the actin cytoskeleton is involved in both host resistance, and pathogen virulence.

PS01-055

Defense-related WRKY transcription factors respond to components of the plant cell wall in *Arabidopsis*

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Several WRKY transcriptions factors are associated with defense against fungal pathogens and response to chitin. Among these, we indentified a subset that also responds to components of the plant cell wall. Recent literature suggests an interplay between the genetic regulation of defense response and monitoring of plant cell wall integrity, analogous to yeast cell wall integrity signaling (CWI). We hypothesize that the plant cell monitors cell wall damage caused by both mechanical and biochemical changes that occur during pathogen invasion, and that some of the signaling molecules involved in cell wall surveillance are common to those found in pattern triggered immunity (PTI). Our survey identified cellodextrins and pectin oligos as triggering *WRKY30* and *WRKY40* transcription, by both promoter::GUS fusion lines and Q-PCR. In time-course experiments of cellobiose response, we observed that gene expression peaked at 25 minutes post-treatment. *WRKY30* was the most strongly up-regulated, with expression increasing by over 100 fold. Additionally, *WRKY30p::GUS* data revealed that every cell type in seedling roots responds to cellobiose treatment. We are employing a yeast-one hybrid approach using cDNA library generated from seedling roots treated with cellobiose to look for proteins that bind to the WRKY30 promoter. We also performed a microarray experiment using seedling roots treated with various cell wall components. We believe that this combined approach will help elucidate the sensing/signaling cascades involved in the response to cellobiose, and more broadly, the existing mechanisms that mediate signals derived from perturbations of the cell wall.

PS01-056

Identification of signalling partners and internalization regulators of FLS2 by mass spectrometry

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Plants perceive conserved pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) through plasma membrane-localized receptors referred to as pattern recognition receptors (PRRs). Signalling events triggered by PRRs result in increased plant defences against potential invasive pathogens, a phenomenon known as PAMP-Triggered Immunity (PTI). Several PRRs have been described and among them, FLAGELLIN SENSING 2 (FLS2), from *Arabidopsis thaliana*, is one of the best characterized and confers resistance to bacterial infection through the recognition of bacterial flagellin (flg22). After perception of flg22, FLS2 relocates from the plasma membrane and is internalized within minutes. Functions of this internalization remain poorly understood.

To identify new FLS2 partners that participate in internalization of the receptor and/or signalling, we undertook large scale FLS2-GFP pull-down assays before and after flg22 treatments followed by mass spectrometry analyses. In addition to the known SERK3 and SERK4 co-receptors, this approach allowed us to identify several proteins with putative functions in signal transduction and trafficking. Taking advantage of *Nicotiana benthamiana*, where PRR internalization can be observed after ligand application (see Gervasi et al., Poster XV MPMI), function of selected candidates in FLS2 internalization is now assessed by transient expression. Our goal is to genetically block FLS2 internalization to help deciphering the interception between FLS2 internalization and FLS2 signalling.

PS01-057

Flagellin and the role of the *Pseudomonas syringae* type III secretion system in eliciting and suppressing immune responses independent of effectors

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Bacterial flagellin is perceived as a microbe-associated molecular pattern (MAMP) by the extracellular pattern recognition receptors, FLS2 and TLR5, of plants and mammals, respectively. Flagellin translocated into mammalian cells by pathogen type III secretion systems (T3SSs) induces an NLRC4-dependent, death-associated immune response. We are investigating the ability of the *P. syringae* T3SS to elicit and suppress immune responses in *Nicotiana benthamiana* using several bacteria: *Pseudomonas fluorescens* Pf0-1 expressing a *P. syringae* T3SS, an effectorless *P. syringae* pv. *tomato* DC3000 polymutant, and a variety of Pf0-1 and DC3000 derivatives deficient in flagellar biogenesis and T3SS secretion/translocation functions. Flagellin (FliC) was secreted in culture and translocated into plant cells by the T3SS expressed in Pf0-1 and DC3000 mutants. Pf0-1 and DC3000 *fliC* mutants were strongly reduced in functional immunity elicitation as indicated by the ability of challenge-inoculated bacteria to translocate the AvrPto-Cya effector-reporter hybrid and other assays. Elicitation of immune responses was partially restored to FiC^{+} *flgGHI* flagellar pathway mutants by wild-type and translocation-deficient (secretion-proficient) T3SSs. *Agrobacterium*-mediated transient expression in *N. benthamiana* of FliC with or without a eukaryotic export signal peptide, coupled with virus-induced gene silencing of FLS2, revealed no death response or any immune response that was not FLS2 dependent. FliC alters immune responses elicited by a subset of type III effectors, and FliC-elicited immune responses in *N. benthamiana* are partially suppressed by a DC3000 T3SS component. Our findings reveal interplay between FliC and the T3SS and a major difference in the immune systems of plants and mammals.

PS01-058

Role of the *HaHOG1* MAP kinase in response of the conifer root and but Rot pathogen (*Heterobasidion annosum*) to osmotic and oxidative stress

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The basidiomycete *Heterobasidion annosum* (Fr.) Bref. *s.l.* is a filamentous white rot fungus considered to be the most economically important pathogen of conifer trees. Despite the infection severity, very little is known about the molecular and biochemical aspects related to adaptation to abiotic stresses. In this study, the osmotic and oxidative tolerance and the role of the *HaHOG1* Mitogen Activated Protein Kinase (MAPK) gene was investigated. The transcript levels of selected genes known to have an important role in osmotolerance were also quantified under osmotic conditions. The *HaHOG1* gene was used for an heterologous expression and functional study in the *Saccharomyces cerevisiae hog1* mutant strain. Moreover, the phosphorylation level of HaHog1p was studied under osmotic and oxidative stress. The results showed that *H. annosum* displayed a decreased growth when exposed to an increased concentration of osmotic and oxidative stressors. Among the genes studied, *PMC1* was highly induced when the fungus was exposed to $CaCl_2$ for 60 minutes. The *HaHOG1* gene was able to restore the osmotolerance and oxidative tolerance in the *S. cerevisiae hog1* mutant strain. The HaHog1p was strongly phosphorylated in the presence of NaCl, KCl, hydrogen peroxide but not in the presence of $CaCl_2$ and $MgCl_2$. Finally, the GFP-HaHog1p fusion protein accumulated in the nuclei of the *S. cerevisiae hog1* mutant cells when exposed to high osmotic conditions. Taken together these results provide the first insights about the response of *H. annosum* to osmotic and oxidative stress and elucidate the role of the *HaHOG1* gene in such conditions.

PS01-059

Interplay between two Arabidopsis genes, *NHR1A* and *NHR1B*, regulating stomatal defense and nonhost disease resistance against bacterial pathogens

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Nonhost disease resistance (NHR) is the most common form of plant defense mechanism exhibited by all plants towards the majority of plant pathogens. We have used a virus-induced gene silencing-based fast forward genetics screen in *Nicotiana benthamiana* to identify plant genes that play a role in NHR. Using this approach we have identified several genes that play a role in NHR. One of these genes encodes a GTP-binding protein and silencing of this gene in *N. benthamiana* compromised NHR. To further characterize the gene function, we identified two Arabidopsis homologs, *NHR1A* (NHR-associated gene 1A) and *NHR1B* (NHR-associated gene 1B) that regulate stomatal defense and NHR against bacterial pathogens, respectively. Arabidopsis *nhr1a* mutant is impaired in stomata closure in response to ABA, flg22, LPS, and nonhost bacterial pathogens, indicating that *NHR1A* acts as a positive regulator of stomata closure in response to both abiotic and biotic stresses. By contrast, the down-regulation of *NHR1B* using RNA interference (RNAi) was not disrupted in ABA, PAMPs, and nonhost pathogen induced stomatal closure. However, interestingly, NHR was compromised in *NHR1B* RNAi lines. Thus, our findings in the present study indicate the complex interplay between two novel Arabidopsis genes, *NHR1A* and *NHR1B*, in the regulation of stomatal defense and NHR against bacterial pathogens. We will further discuss the possible functions and mechanisms of NHR1A and NHR1B involved in NHR against bacterial pathogens.

PS01-060

Does the Arabidopsis endogenous peptide elicitor/receptor Pep/PEPR pathway act in danger sensing and signalling in plant immunity?

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Recognition of the so-called microbe-associated molecular patterns (MAMPs), such as bacterial flagellin or elongation factor Tu, triggers immune response that restricts multiplication of potentially infectious microbes. In Arabidopsis, recognition of the endogenous elicitor epitopes Pep1-Pep6 triggers immune response likewise. The conserved Pep epitope is embedded in the C-termini of the precursors PROPEPs which lack a canonical N-terminal signal peptide for entering the secretory pathway, but is recognized by the extracellular domain of the trans-membrane receptors PEPR1 and PEPR2. This implies a model in which cellular damages, *e.g.* upon pathogen challenges, expose the elicitor-active epitope to PEPRs

in the extracellular spaces, thereby triggering immune signalling. However, how this recognition occurs remains to be shown in vivo. Of 6 Arabidopsis PROPEPs, we focus on PROPEP2 and PROPEP3 that are strongly induced upon pathogen-derived molecules at the mRNA level. We undertake a biochemical approach to trace the actions of the two PROPEPs at the protein level in vivo. Our data indicate that both PROPEPs accumulate upon MAMP application and that they generate active ligands that bind to PEPRs and induce the recruitment of the co-receptor BAK1 to PEPRs. We are currently testing whether PROPEP processing and/or translocation contributes to the generation of elicitor-active ligand(s) for PEPRs and whether this process is influenced upon pathogen challenges. We will discuss whether, and if so, how this endogenous elicitor system serves in damage sensing and signaling during immune response.

PS01-061

A classification tool for calcium dependent protein kinases (CPKs) based on motif analysis

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Calcium dependent protein kinases (CPKs) are a unique gene family found in plants, algae and protists. CPKs respond to a range of abiotic and biotic stressors, including infection by various fungal, bacterial and viral pathogens. CPKs are currently identified either individually or from whole-genome sequences and classified into one of six evolutionary groupings. This classification is based on either sequence homology with previously reported CPKs or phylogenetic analysis - usually with sequences from *Arabidopsis thaliana*. This approach requires time, familiarity with bioinformatics software and familiarity with CPK evolutionary groups. Our aim was to develop a simpler motif-based tool to classify newly identified CPKs quickly into their corresponding evolutionary groups, which will complement phylogenetic analysis. To produce this tool we performed three steps. Firstly we aligned the protein sequences of all 34 CPKs from *A. thaliana* (AtCPKs), and identified 64 motifs, 11 conserved and 53 that vary between evolutionary groups. Secondly, we assessed these AtCPK motifs using a training set of CPK genes from the rice, potato and grape genomes. Thirdly, discriminative motifs were validated using CPK sequences from three complete genomes (wheat, poplar and sorghum). The utility of the CPK-motif classification tool was evaluated with a testing set of CPKs from both complete and incomplete genomes, including kiwifruit. These discriminative motifs can facilitate CPK gene prediction and may also prove useful as targets for degenerate primers, allowing for extensive discovery of CPK orthologues from diverse plant species (for which genome sequences are unavailable) that respond to different types of pathogenic infection.

PS01-062

Molecular characterization of wound-inducible MAPK cascade in rice

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Mechanical wounding in plants induces dramatic changes in gene expression and protein activation that contribute not only repairing of damaged plant tissue but also participating in the activation of wound defense signaling pathways. To understand the signaling pathway of wounding in rice, we have investigated the involvement of protein kinase. The rice 48-kDa MBP kinase <named OsSIPK> was rapidly activated within 10 min of wounding. In order to characterize the upstream kinase of OsSIPK in wounding signaling pathway, we used Agrobacterium-mediated transient expression system in tobacco. Transiently expressed $OsMKK4^{DD}$, a constitutively active mutant of OsMKK4, induced HR-like cell death and showed high level activation of endogenous MAPKs in tobacco. These results strongly suggest that OsMKK4 is the functionally interchangeable with NtMEK2 in tobacco. Furthermore, $HisOsMKK4^{WT}$ and $HisOsMKK4^{DD}$ phosphorylated $HisOsSIPK^{KR}$ but not $HisMPK4^{KR}$ or $HisBWMK1^{KR}$, two other rice MAPKs. Phosphorylation of HisOsSIPK by the $HisOsMKK4^{WT}$ and $HisOsMKK4^{DD}$ enhanced their activities toward myelin basic protein as a substrate. Expression of $OsMKK4^{DD}$ activates OsSIPK after wounding, suggesting that the OsMKK4 is an upstream kinase of OsSIPK in wounding signaling pathway in rice.

PS01-063

Different receptor systems regulate chitin signaling in *Arabidopsis* and rice

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Plants and animals recognize microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) for sensing of microbes. Chitin derived from fungal cell wall is a typical MAMP and its perception leads to immune responses. CEBiP (rice) and CERK1/OsCERK1 (*Arabidopsis*/rice) have been identified as critical components for chitin signaling in these plants (1, 2, 3). They are a GPI-anchored protein and a receptor-like kinase (RLK), respectively. To understand whether *Arabidopsis* requires the presence of CEBiP-like molecule(s) for chitin signaling, we characterized CEBiP homologues in *Arabidopsis*. One of three CEBiP homologues (AtCEBiP) showed a high-affinity binding for chitin oligosaccharides using affinity labeling with biotinylated chitin oligosaccharides (4). The binding characteristics of AtCEBiP were very similar to rice CEBiP. However, the knock-out mutant as well as overexpressing plant of AtCEBiP showed chitin-induced ROS generation similar to wild type *Arabidopsis*. These results indicated that AtCEBiP is biochemically functional as a chitin binding protein but does not significantly contribute to signaling. In other words, only AtCERK1 seems enough for chitin perception and signaling in *Arabidopsis*. Thus, the machinery required for chitin perception/signaling in *Arabidopsis* seems to be significantly different from that of rice, which requires both CEBiP and OsCERK1.
(1) Kaku et al., Proc Natl Acad Sci U S A. 2006, 103:11086-91; (2) Miya et al., Proc Natl Acad Sci U S A. 2007, 104:19613-8; (3) Shimizu et al., Plant J. 2010, 64:204-14; (4) Shinya et al., Plant Cell Physiol. 2010, 51:262-70.

PS01-064

Phosphorylation of *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defense response

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Mitogen-activated protein kinase (MAPK) cascades have pivotal roles in plant innate immunity. However, downstream signaling of plant defense-related MAPKs is not well understood. Here we provide evidence that *Nicotiana benthamiana* WRKY8 transcription factor is a physiological substrate of SIPK, NTF4, and WIPK. Clustered proline-directed serines (SP cluster), which are conserved in group I WRKY proteins, in the N-terminal region of WRKY8 were phosphorylated by these MAPKs in vitro. Anti-phosphopeptide antibodies indicated that serines in the SP cluster

of WRKY8 are phosphorylated by SIPK, NTF4, and WIPK in vivo. The interaction of WRKY8 with MAPKs depended on its D domain, which is a MAPK-interacting motif, and this interaction was required for effective phosphorylation of WRKY8 in plants. Phosphorylation of WRKY8 increased its DNA-binding activity to the cognate W-box sequence. The phospho-mimicking mutant of WRKY8 showed higher transactivation activity, and its ectopic expression induced defense-related genes, such as *3-hydroxy-3-methylglutaryl CoA reductase 2* (*HMGR2*) and *NADP-malic enzyme* (*NADP-ME*). In contrast, silencing of *WRKY8* decreased the expression of defense-related genes and increased disease susceptibility to plant pathogens. Thus, MAPK-mediated phosphorylation of WRKY8 has an important role in the defense response through activation of downstream genes.

PS01-065

Post-translational modification of WRKY transcription factors by MAPKs induces HR-like cell death

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MAPK cascades play pivotal roles in signaling pathway of plant defense. *WRKY8*, group I WRKY transcriptional factor in *Nicotiana benthamiana*, is isolated as a substrate of pathogen-responsive MAPKs, and it has been shown that WRKY8 is specifically phosphorylated by SIPK, NTF4 and WIPK. Proline-directed serine (SP cluster), which is existed in N-terminal region of WRKY8, is target of phosphorylation. Phosphorylation of WRKY8 increases DNA binding and transcriptional activities. Additionally, WRKY8 contains D domain which is a MAPK-interacting motif, and D domain-dependent interactions with MAPKs are required for effective phosphorylation of WRKY8. Interestingly, SP cluster and D domain are highly conserved in some members of group I WRKYs. To identify novel substrates of MAPKs, we isolated seven novel group I *WRKY* genes from cDNA library of *N. benthamiana*, and these *WRKY* genes carry SP cluster and D domain. Transient expression of four novel *WRKY* genes induced cell death in *N. benthamiana* leaves. WRKY-dependent cell death was accelerated by phospho-mimicking mutation in putative phosphorylated serines of SP cluster. Moreover, $MEK2^{DD}$-dependent HR-like cell death was compromised in multiple *WRKY* genes-silenced plants. These results suggest that WRKY transcriptional factors are involved in the regulation of cell death at the downstream of MAPK.

PS01-066

Identification of flagellin receptor in rice involved in induction of plant immune responses

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Flagellin from rice-avirulent strain of phytopathogenic bacteria *Acidovorax avenue* induces several immune responses in rice. In *Arabidopsis*, the most conserved N-terminal domain of flagellin that consists of a 22-amino acid peptide (flg22) was recognized by a receptor-like kinase, Flagellin Sensing 2 (FLS2). Although, rice possesses FLS2 ortholog (OsFLS2), rice immune responses were not induced by flg22. This indicates that rice has a different receptor from FLS2 for recognition of the flagellin from rice-avirulent strain of *A. avenae*. To clarify the flagellin recognition mechanism in rice, we attempted to identify the receptor for the flagellin in rice. Gene expression profiling in rice after the flagellin treatment showed that expression of several genes encoding the receptor kinase were increased. The rice T-DNA insertion mutant of one identified gene, *flagellin-induced receptor kinase 2* (*flirk2*), lost the ability of the flagellin recognition. Expression of *FliRK2* in *flirk2* mutant was recovered the induction ability of several immune responses, such as H_2O_2 generation and immune related gene expression by flagellin treatment, while the expression of the kinase domain deleted *FliRK2* in *flirk2* did not show the recovery such induction ability of immune responses. These results suggest that FliRK2 transduces flagellin recognition signal through the protein phosphorylation into the cell.

PS01-067

An interaction-based identification of MKK3 upstream factors in *Arabidopsis*

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In plants, mitogen-activated protein kinase (MAPK) cascades are involved in various biotic and abiotic stress responses. Recent studies showed that Arabidopsis MKK3 has multiple functions with different downstream MAPKs. The MKK3-MPK6 cascade negatively regulates JA signaling. The MKK3-MPK1/2/7 cascade participates not only in defense responses but also in regulation of ABA and salt signaling. The MKK3-MPK8 cascade has a function in part of regulation ROS (reactive oxygen spices) homeostasis. However, upstream MAPKKKs of these cascades still remain to be identified. Considering case of existing MAPK cascade, we assumed that protein-protein interaction could be an important tool for identification of MKK3 upstream factors. With the concept, we performed systematic yeast two-hybrid analysis using Arabidopsis 21 MAPKKKs, 8 MAPKs and MKK3. We found that 4 MTKs (MKK3-interacting MAPKKKs) bound to MKK3. Among of these, only MTK1interacted with MPK6. We also detected MKK3-MPK1/2/7 interaction. These results suggest presence of two signaling modes, MTK1-MKK3-MPK6 and MTK1/2/3/4-MKK3-MPK1/2/7. In the former case, MTK1 may serve a scaffold function, not only as a MAPKKK, to establish specific cascade. This hypothetical cascade fits to the JA signaling model. The latter case may have roles in ABA and salt signaling.

PS01-068

Functional analysis of LysM motifs in rice chitin receptor CEBiP

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Plants are equipped with a sensitive system to detect Microbe/Pathogen-associated molecular patterns (MAMPs/PAMPs) and initiate various defense responses. Chitin (β-1,4-linked polymer of *N*-acetylglucosamine) is a common component of the cell walls of various fungi. Fragments of chitin, *N*-acetylchitooligosaccharides, are one of the major MAMPs and have been shown to act as a potent elicitor signal in wide range of plant systems. Recently we identified two LysM receptors, CEBiP and OsCERK1, which play an important role for chitin signaling in rice (1, 2). CEBiP is a GPI anchored protein and has several LysM motifs in the extracellular domain, which seem to contribute for chitin binding activity. To investigate the role of these LysM motifs in sugar binding specificity of CEBiP, we applied two different approaches, deletion of each LysM motif from CEBiP and replacement of these LysM motifs with those of CEBiP homologues with/without chitin binding activity. We found that one of the motifs plays a critical role for chitin binding in CEBiP. (1) Kaku et al, *PNAS*, 103, 11086 (2006); (2) Shimizu et al, *Plant J.*, 64, 204 (2010).

PS01-069

Identification and localization of the NB-LRR gene family in the hot pepper genome (*Capsicum annuum*)

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A sessile organism, plants constitutively challenged with pathogens have been developed various strategies for protection, such as innate immune receptors and disease resistance R genes. As the largest and most variable gene families in plant genomes, the nucleotide binding and leucine-rich repeat (NB-LRR) encoding resistances genes play key roles in defense and surveillance against pathogens and pest. The NB encoding R gene family has been studied extensively to unravel the role of their function and evolution in plants. Here, the diversity of NB-LRR genes was investigated in the Capsicum annuum CM334 draft genome sequence. We performed a conserved NB domain based search of the annotated pepper genome and identified 486 NB-LRR type genes among the 34,534 pepper gene models. Based on our pipeline, 23 were TNL type contains an N-terminal toll/interleukin 1 receptor (TIR)-like domain, and 463 were non-TNL type contain an N-terminal coiled-coil (CC) domain or not. The transcript levels of 443 NB-LRR genes were detected from variable tissues. The physical and genetic map contributions were positioned for these NB-LRR genes in pepper pseudo-molecule chromosomes. Comparative genomics studies on evolution and function of NB-LRR genes in Solanaceous genomes is on progress. These will provide a major source of candidates for improving defense mechanism and understanding R gene evolution and diversity in pepper and other Solanaceae species.

PS01-070

Discovery of a small peptide that can activate the plant immune system from combinatorial random peptide libraries

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Plants defend themselves using an innate immune system, which is activated in response to a variety of molecules derived from pathogens. These molecules have provided profound insight into mechanisms of pathogen recognition and subsequent signaling pathways in plants. In the present study, we screened a combinatorial random hexapeptide library for peptides that can activate the plant immune system using a cell-based high-throughput screening system, in which H_2O_2 generation was monitored. We successfully discovered a novel hexapeptide (YGIHTH-amide, PIP-1) from the random library prepared by split-mix synthesis, which triggered an oxidative burst in tobacco and tomato cells at low micromolar concetrations, but not in Arabidopsis cells. Interestingly, PIP-1 shares no sequence similarity to any known peptide elicitors derived from pathogens. PIP-1 also induced significant levels of phytoalexin biosynthesis. From analysis of defense-related gene expression in tobacco cells, PIP-1 is likely to activate the immune system via a jasmonic acid pathway. We also investigated the structural factors important for activity of PIP-1. Alanine-scanning experiments revealed that the N-terminal 4 residues were essential for induction of an oxidative burst. In addition, when the C-terminal amide was converted to acid (PIP-1-OH), an 8-fold increase in activity as compared with PIP-1 was observed. This indicates that the free acid structure at the C-terminus is favorable for activity. PIP-1 is not only useful as a chemical probe for better understanding the plant immune system, but also can serve as a lead compound for the development of new activators of plant defenses.

PS01-071

Arabidopsis ubiquitin ligase ATL31 which is involved in defense response ubiquitinates 14-3-3 proteins in phosphorylation-dependent manner

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Plants sense balance of carbon (C) and nitrogen (N) metabolites to regulate metabolism and development, called C/N response. We previously revealed that ubiquitin ligase ATL31 functions in the C/N response in Arabidopsis (Sato et al., Plant J., 2009). In addition, we demonstrated that the ATL31 is also involved in defense response (Maekawa et al., Plant Mol. Biol., 2012). However, these molecular mechanisms remain unclear. The 14-3-3 proteins were identified as an ubiquitination target of the ATL31 by proteomic approach (Sato et al., Plant J., 2011). 14-3-3 proteins play an important role in regulation of key enzymes involved in C and N metabolisms and defense response. Generally, 14-3-3 proteins bind to phosphorylated motifs in their target proteins. Four putative 14-3-3 binding sites on ATL31 were identified by Scansite search. We determined whether these sites are critical for the 14-3-3 interaction by substitution analysis. The mutated ATL31 which is abolished for phosphorylation was not able to bind to 14-3-3 proteins. Furthermore, phosphorylated peptides generated after the ATL31 protein inhibited interaction between the ATL31 and 14-3-3 proteins. Taken together, these results indicate that the binding activity of 14-3-3 proteins dependent on the phosphorylation status on the ATL31 protein.

PS01-072

Towards understanding MAPK cascade function in potato-PVY interaction

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Sequential activation of kinases within the mitogen-activated protein (MAP) kinase (MAPK) cascades is a common and evolutionary-conserved mechanism of signal transduction. In the last decade numerous reports have provided evidence for the involvement and importance of MAPKs in regulating plant innate immune responses. However the mechanisms through which MAPKs transduce the signals are largely unknown. To gain insight into their potential relationship we are optimizing a yeast two hybrid based screening system. Potato MAPKs from each of the three sequentially phosphorylating and activating components (MAPK, MAPKK and MAPKKK), previously identified to be involved in the potato virus Y response, were selected. They are subjected to screening potato cDNA library. In parallel protein-protein interactions are being verified *in planta*. The data should contribute to better understand the complex network of plant defense signaling pathways.

PS01-073

Two U-box ubiquitin ligases positively contribute to MAMP-responsive MAP kinase cascade in *Arabidopsis*

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Plants primarily recognize microbial pathogens through perception of microbe-associated molecular patterns (MAMPs) by pattern-recognition receptors (PRRs) in the plasma membrane. Perception of MAMPs induces several molecular responses such as defense gene expression, phytoalexin biosynthesis, and cell wall crosslinking, leading to the MAMP-triggered immunity (MTI). MTI contributes to broad-spectrum resistance. This innate immunity is thought to be regulated by mitogen-activated protein kinase (MAPK) cascades. Of those, an *Arabidopsis* MAPK cascade consisting of MEKK1 - MKK1/MKK2 - MPK4 regulates innate immunity signaling. Regulatory mechanism of MEKK1, however, remains to be elucidated. Here, we identified two U-box ubiquitin ligases, PUB25 and PUB26, as MEKK1-interacting proteins by the yeast two-hybrid screening. These proteins specifically interacted with the N-terminal regulatory domain of MEKK1.To see function of PUB25 and PUB26 in the MEKK1-mediating MAP kinase cascade, we produced *pub25/pub26* double mutant and PUB26 overexpressors. We observed reduced MPK4 and MPK6 activation by flg22 in the *pub25/pub26* double mutant. Moreover, *PAD3* induction by flg22 was also reduced in the double mutant. By contrast, *PUB26* over expressors demonstrated higher *PAD3* expression than wild type. These results suggest that positive contribution of PUB25 and PUB26 to MEKK1 function in Arabidopsis.

PS01-074

Visualisation of lateral plasma membrane segregation and phosphorylation-dependent dynamics of remorin proteins in *Arabidopsis thaliana*

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Plasma membranes (PMs) require high levels of plasticity to modulate perception and transduction of signals. While the plant cell wall represents a physical and mostly unspecific barrier for invading microbes, the PM is at the forefront of microbial recognition and initiation of defence responses. Accumulating evidence indicating dynamic compartmentalization of PMs in response to environmental cues has evoked increasing interest in its compositional heterogeneity. Remorin proteins are PM localised plant specific proteins involved in signalling during plant microbe interactions. Recently symbiosis specific remorins from *Medicago truncatula* and *Lotus japonicus* as well as a remorin from potato have been shown to interact with and being phosphorylated by key components of plant-microbe signaling pathways. Both proteins are crucial for the corresponding pathways and serve as markers for so called membrane rafts, sterol rich compartments in the PM that are believed host a number of signalling proteins but also serve as key cellular entry points for pathogenic microbes and viruses. In a global approach we determined the sub-cellular localization of 15 out of 16 remorins from *Arabidopsis thaliana*. We observed high degrees of lateral segregation of the plasma membrane, with different remorin family members labelling approximately 10 discriminative membrane domains. Some of these compartments are highly dynamic, laterally relocalising upon stress treatment in a phosphorylation dependent manner and fusing to form larger platforms.

PS01-075

Towards the identification of NFBS2: a high affinity Nod Factor Binding Site in *Medicago truncatula* cell suspension cultures

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Nod factors are lipo-chitooligosaccharides (LCOs) involved in the specific recognition of the bacterium by the plant during the establishment of the legume-*Rhizobium* symbiosis. By using radiolabelled Nod factors, we have been able to characterise Nod factor-binding sites (NFBSs), in *Medicago spp*. One of these binding sites, termed NFBS2, is associated to the microsomal fraction of *Medicago truncatula* cell suspension cultures and exhibits a high affinity for the major Nod factor produced by *Sinorhizobium meliloti*, the symbiont of *M. truncatula*. NFBS2, which does not correspond to the putative Nod factor receptors NFP or LYK3, is specific for the LCO structure since it recognizes recently identified Myc-LCOs as well as Nod factors with a high affinity, but not chitooligosaccharides. NFBS2 discriminates the length of the fatty acid, the degree of polymerisation of the oligochitin backbone, but not the sulfate group that is the main determinant of the specific interaction between *S. meliloti* and *M. truncatula*. Because of its interesting characteristics in terms of LCO recognition we are attempting to identify NFBS2. We first developed LCO-derivatives in order to detect the binding protein by photoaffinity labelling in microsomal preparations. Then, by exploiting the difference of abundance of the binding protein in different cell lines, we have combined proteomic and transcriptomic approaches to identify proteins/transcripts that could correspond to NFBS2 according to their relative abundance in these lines. The on-going work will be presented and discussed.

PS01-076

The binding affinity to viral coat proteins determines the recognition specificity of allelic L tobamovirus resistance proteins

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Alleles of Capsicum L gene, L^1, L^2, L^3, and L^4, confer broadening spectrum of tobamovirus resistance accompanied by hypersensitive response (HR). Their protein products consisting of three domains (coiled-coil, nucleotide-binding and leucine-rich repeat (LRR) domains) recognize tobamovirus coat proteins (CP) with different specificities determined by the LRR domain. The allelic L proteins, L^1, L^2, L^3, and L^4, showed increasing binding capacity to different tobamovirus CPs, suggesting the correlation between the binding affinity and CP recognition by L proteins. To confirm this notion, we performed mutational analysis and identified some xxLxLxx beta-sheet motifs responsible for the recognition. The substitution to xALALAx of 12th or 32th motifs narrowed the recognition spectrum and the combination of the mutations narrowed the spectrum further. The single- and double-mutants recognized *Tomato mosaic virus* (ToMV) CP to decreasing extents manifested by delayed HR, and exhibited decreasing binding affinity to ToMV CP. These results suggest that the binding affinity between L and CP (or the stability of the complex containing these proteins) is important for generating resistance signals, although it remains unknown whether these proteins interact directly or indirectly to each other. Although the amino acid sequences of the LRR domain determine the affinity between L and CP, either the LRR domain or the N-terminal domains, when expressed alone, did not interact with CPs. We will discuss the formation of the recognition complex based on the analyses of recognition-defective L protein mutants

and recently identified L-interacting proteins.

PS01-077

Molecular analysis of *Pia*-mediated resistance, regulated by a pair of NB-LRR proteins

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In rice, *Pia* disease resistance locus confers race specific resistance against rice blast fungus Magnaporthe oryzea when it contains *avrPia*. Recent study demonstrated that *Pia* locus consists of two kinds of nucleotide-binding leucine rich repeat domain (NB-LRR) genes, *RGA4* and *RGA5*. They are both necessary to recognize *avrPia*. Classical studies hypothesized that relationship between one plant disease resistance gene and one pathogen avirulence gene regulate a race specific disease resistance. However, some researchers reported that a pair of NB-LRR proteins mediate race specific resistance, although their biochemical relationship is largely unknown. We tried to analyze the relationships among RGA4, RGA5 and avrPia to understand the molecular mechanism of *Pia*-mediated resistance. In transient overexpression in *Nicotiana benthamiana*, RGA4 induced the hypersensitive response (HR) -like cell death and ROS production. Co-expression of *RGA5* and *RGA4* suppressed this phenotype. Localization analysis of fluorescence-protein tagged-RGA4, -RGA5 and avrPia revealed that these proteins mainly accumulated in the cytoplasm and/or in the cytoplasm and the nucleus in rice protoplasts. Co-immunoprecipitation assay showed that RGA4 and RGA5 form a complex. Taken together, these results suggested that RGA4 and RGA5 interact in the cytoplasm and the RGA4 function is suppressed by RGA5. Once avrPia is recognized by RGA4-RGA5 complex within the cell, *Pia*-mediated resistance is activated.

PS01-078

A new family of endogenous peptide elicitors conserved in Fabales and Cucurbitales

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Plant endogenous peptide elicitors are newly emerging bioactive peptides that are involved in defense responses against pathogens and herbivores. Five different kinds of endogenous peptide elicitors, systemin, hydroxyproline-rich systemin, plant elicitor peptide (Pep), a peptide derived from subtilase (GmSubPep), and inceptin, have been identified thus far. Recently we isolated a novel eight-amino acid peptide from soybean leaves by monitoring medium alkalinization activity, and named it GmPep914 based on its molecular mass. The amino acid sequence of GmPep914 is completely different from known endogenous peptide elicitors. Soybean genome database analysis revealed a similar peptide, GmPep890. The addition of synthetic GmPep914 and GmPep890 into soybean suspension cultured cells induced medium alkalinization and induced the expression of defense-related genes at nanomolar concentrations. The expression levels of the precursor protein genes, *GmPROPEP914* and *GmPROPEP890*, were extremely high in roots and were induced by salicylic acid, jasmonic acid and ethylene in leaves. Analysis of the plant genome database revealed that other leguminous plants contain similar sequences, and two similar sequences in cucumber. Synthetic cucumber peptides inhibited root growth, another characteristic of defense elicitors. Interestingly, the expression of the precursor protein genes of the cucumber peptides is very high in roots, similar to the soybean peptides. The data suggest that GmPep914-type endogenous peptide elicitors are conserved within the Fabales and Cucurbitales.

PS01-079

Elucidation of the defensive role of GmPep914 and Gmpep890 in soybean plant

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Recently, an eight-amino acid peptide, GmPep914, was isolated from soybean leaves as a medium alkalinization factor, and its homolog, GmPep890, was predicted from the soybean genome database. Both GmPep914 and GmPep890 are considered to be endogenous peptide elicitors based on their abilities to induce the expression of defense-related genes in soybean suspension cultured cells. However, it is not clear whether GmPep914 and GmPep890 are involved in defense responses in soybean plants. Here, we elucide the physiological role of GmPep914 and GmPep890 in defense responses in soybean leaves. We developed a method for supplying peptides to soybean leaves through cut petioles without activating wound responses. Both GmPep914 and GmPep890 induced the expression of defense-related genes including *GmCYP93A1*, *GmChi1b-1*, and *Gmachs1* with maximum induction after 8 hours. The precursor protein genes of *GmPep914* and *GmPep890*, *GmPROPEP914* and *GmPROPEP890*, respectively, were also induced by GmPep914, GmPep890 and elicitors from pathogens. A one nM solution of the peptides was sufficient for induction: comparable to other endogenous peptide elicitors and peptide hormones. It has been reported that the transcripts of *GmPROPEP914* and *GmPROPEP890* accumulate preferentially in roots. We found that root preferential accumulation is not dependent on soybean varieties or growing conditions. Promoter regions of *GmPROPEP914* and *GmPROPEP890* are rich in cis-elements for defense responses and for root specific expression. Combined results suggest that GmPep914 and GmPep890 function as endogenous peptide elicitors with a special role in roots. Further experiments to confirm these observations are currently in progress.

PS01-080

Characterising endosomal proteomes during defence responses

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The first active innate immune response is triggered as pathogens access the plant interior and encounter extracellular surface receptors. Subcellular trafficking plays a critical role in several steps of receptor function from their biogenesis, glycosylation and insertion into the plasma membrane, to their specific location through constitutive recycling and finally to their destruction after endocytosis. Endocytosis of active receptor complexes is likely to play a role in signal attenuation, and may also contribute to signal propagation. We seek to identify proteins associated with various endosomal compartments in Arabidopsis thaliana seedlings both before and after elicitation of the Flagellin Sensing 2 receptor (FLS2) and will discuss the differences in these sub-proteomes.

PS01-081

Quantitative proteomics reveals dynamic changes at the plasma membrane during *Arabidopsis* immune signaling

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Many classes of plant pathogens remain outside the host cell membrane during their lifecycle. As a result, the plant plasma membrane (PM) mediates critical aspects of plant immunity including pathogen recognition, signal transduction, and downstream defense responses. Investigating how the plasma

membrane proteome changes during these events will lead to a better understanding of plant immune signaling and identify novel components of plant disease resistance. We have used label-free shotgun proteomics to examine PM dynamics during plant defense signaling. Transgenic Arabidopsis plants expressing the bacterial effector AvrRpt2 under the control of a dexamethasone (Dex)-inducible promoter were used to initiate effector-triggered immunity (ETI). Expression of the AvrRpt2 protease results in RIN4 cleavage and activation of the disease resistance protein RPS2. PM vesicles were isolated 6 hours post-Dex treatment and subjected to gel-enhanced liquid chromatography tandem mass spectrometry (Gel LC-MS/MS) for protein identifications. More than 2300 proteins were identified in total and label-free spectral counting was employed to quantify relative protein abundance. Over 20% of upregulated proteins have known roles in plant immune responses. Proteins that are up-regulated during ETI include those involved in calcium and lipid signaling, membrane transport, metabolism, protein phosphorylation, redox homeostasis, and vesicle trafficking. A similar approach is being undertaken to examine pattern-triggered immune (PTI) responses upon activation of the FLS2 immune receptor. Preliminary data indicate that activation of ETI and PTI results in distinct, yet overlapping, patterns of PM protein regulation. These experiments provide a framework for understanding global PM proteome dynamics during plant immune responses.

PS01-082

FLS2/BIK1/BAK1 association and dissociation are not sufficient to activate *Arabidopsis* immunity but FLS2 phosphorylation site Ser-938 is required

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FLAGELLIN-SENSING 2 (FLS2) is a leucine-rich repeat/transmembrane domain/protein kinase (LRR-RLK) that is the plant receptor for bacterial flagellin or the flagellin-derived flg22 peptide. Previous work has shown that after flg22 binding, FLS2 releases BIK1 kinase and homologs, and associates with BAK1 kinase, and that FLS2 kinase activity is critical for FLS2 function. However, the detailed mechanisms for activation of FLS2 signaling remain unclear. The present study identified multiple FLS2 in vitro autophosphorylation sites and found that Serine-938 is important for FLS2 function in vivo. FLS2-mediated immune responses are abolished in transgenic plants expressing $FLS2_{S938A}$, while the acidic phosphomimic mutants $FLS2_{S938D}$ and $FLS2_{S938E}$ conferred responses similar to wild-type *FLS2*. FLS2-BAK1 association and FLS2-BIK1 disassociation after flg22 exposure still occur with $FLS2_{S938A}$, demonstrating that flg22-induced BIK1 release and BAK1 binding are not sufficient for FLS2 activity, and that phosphorylation of Ser-938 controls other aspects of FLS2 activity. Purified BIK1 still phosphorylated purified $FLS2_{S938A}$ and $FLS2_{S938D}$ mutant kinase domains in vitro, but $FLS2_{S938A}$ exhibited reduced autophosphorylation activity in vitro and reduced phosphorylation in vivo. Phosphorylation of BIK1 and homologs after flg22 exposure was disrupted in transgenic *Arabidopsis thaliana* plants expressing $FLS2_{S938A}$ or $FLS2_{D997A}$ (a kinase catalytic site mutant), but was normally induced in $FLS2_{S938D}$ plants. Hence FLS2-BIK1 dissociation and FLS2-BAK1 association are not sufficient for FLS2-mediated defense activation, but FLS2 Ser-938 phosphorylation and FLS2 kinase activity are needed both for overall defense activation and for appropriate flg22-stimulated phosphorylation of BIK1 and homologs.

PS01-083

Heterotrimeric G-proteins participate in MAMP-triggered immunity in *Arabidopsis*

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Upon the perception of microbe-associated molecular patterns (MAMPs), activated pattern recognition receptors (PRRs) trigger the signaling pathway leading to MAPK3/6 phosphorylation and subsequent defense gene activation. However, it is unclear how PRRs convey signals to the MAPK pathway and how these signals are translated into the activation of appropriate defense responses. Heterotrimeric G proteins are well-established signaling intermediates in eukaryotes that meditate ligand-recognition signals from transmembrane receptors. Loss-of-function mutants to the canonical β-subunit, AGB1, and a putative β-subunit, AGB2, display altered MAPK signaling when challenged with MAMPs (i.e. bacterial flagellin peptide, flg22, or fungal chitin). This corresponds to defects in receptor dynamics during MAMP elicitation. Based on our results, we can provide a mechanism involving the β-subunit in defense gene activation and that AGB1 acts through the MAPK signaling pathway in bacterial and fungal defense. Our data suggests that the heterotrimeric G-protein complex participates in signal transduction from PRRs to MAPKs, in order to communicate signals that activate appropriate downstream defense responses.

PS01-084

Identification of two *Arabidopsis* glycosyltransferases involved in the perception of MAMPS

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How the perception of different microbes by pattern recognition receptors (PPRs), funneled in conserved MAPK cascade, can lead to pathogen-specific transcriptional profiles remains an open question. The fine tuning of the dynamics of this linear signal transduction by post-translational modifications is one of the means to achieve such specificity. While phosphorylation has been extensively characterized, glycosylation, both O- or N-, catalyzed by glycosyltransferases (GTs), has received little attention. To investigate the role of glycosylation in *Arabidopsis* innate immune response, we screened T-DNA insertion lines in three GT families for an impaired callose response to MAMPS and isolated two mutants, *gmp*(*GT involved in MAMP Perception*)*1* and *gmp2*, that are impaired in the response to flagellin peptide, flg22, and chitin. *gmp2* also shows an altered response to the EF-TU peptide, elf26. Both *GMP*1 and *GMP2* genes are induced upon perception of flg22 and they are required for flg22 mediated protection from *P. syringae* infection and flg22 induced transcriptional response. In addition, *gmp1* displays MAPK phosphorylation, while *gmp2* abolishes it completely indicating that the two GTs act at different levels in regulating the MAMP induced signal transduction. We are currently investigation further the role of GMP1 and GMP2 through biochemical characterization of their enzymatic activity, effects on PPRs localization and biogenesis, and analysis of glycoforms of key proteins in MAMP signal transduction.

PS01-085

N-acyl-homoserine lactone confers resistance toward biotrophic pathogens via altered activation of AtMPK6

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Pathogenic and symbiotic bacteria rely on quorum sensing to coordinate the collective behavior during interactions with their eukaryotic hosts. Many Gram-negative bacteria use N-acyl-homoserine lactones (HSLs) during this communication process. Plants have evolved to perceive HSLs and this perception depends on the length of the acyl moiety and the functional group at the gamma position in the lipid chain of HSLs . Treatment of Arabidopsis roots with the oxo-C14-HSL induces systemic resistance to biotrophic fungi and bacteria Here, we show our

first data on the molecular components of the signaling involved in the response to HSL in plants., Challenging with flg22 of oxo-C14-HSL-treated Arabidopsis plants results in strong activation of mitogen-activated protein kinases AtMPK3 and AtMPK6 and high expression of the defense-related transcription factors WRKY22 and WRKY29 as well as the Pathogenesis related 1 gene. This response was not seen in the mpk6 mutants. Interestingly, not all HSLs induce the observed response in plants. Smaller HSL of the C6 to C10-type do not induce systemic resistance in plants but in contrast exert growth promoting activity.

PS01-086

Mechanism of CDPK function in local and systemic plant innate immune responses

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Calcium-dependent protein kinases (CDPK) are serine/threonine protein kinases, which participate in the activation of environmental biotic and abiotic stress responses. By applying gain- and loss-of-function approaches we characterized distinct CDPK isoforms from *A. thaliana* which became rapidly biochemically activated in response to PAMP-elicitation and trigger the activation of plant defence responses. We combined both transient expression assays and transgenic plant lines expressing wild-type or modified CDPK enzymes. By studying induced responses with respect to protein kinase in vivo activation, we could demonstrate CDPK-specific transcriptional read outs, changes in phytohormone levels and metabolism, and a functional link between PAMP-induced CDPK signalling with enhanced pathogen resistance against infection with *Psydomonas syringae* pv. *tomato* DC3000. In vivo phosphoproteomics combined with enhanced and reduced CDPK signaling not only identified PAMP-induced CDPK phosphorylation targets. Our data also provide evidence how CDPKs are mechanistically involved in the onset of systemic plant defence responses.

PS01-087

Dissection of disease resistance in lettuce using RNAi

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More than 740 candidate genes, potentially involved in pathogen recognition and signaling pathways, have been identified in lettuce. Many of these resistance gene candidates (RGCs) have been mapped relative to 52 disease resistance phenotypes using several approaches. A subset of RGCs, primarily NBS-LRR encoding genes, was selected for functional analysis. RNAi was employed to demonstrate the involvement of candidate genes in determining different disease resistance specificities. Twenty-seven RNAi lines have so far been generated and tested for a total of 23 resistance phenotypes. Thus far, 16 resistance phenotypes in the four Dm3, Dm7, Dm5/8 and Dm13 clusters have been abrogated in different RNAi tester lines. Therefore this strategy can efficiently identify gene families involved in elicitation of disease resistance. In several cases, multiple NBS-LRR encoding genes were involved in a resistance response. RNAi was also used to determine which major signaling pathways are utilized by different resistance genes in lettuce; a dexamethasone-inducible RNAi vector was used because constitutive silencing of *EDS1* and *NDR1* has proven to be lethal in lettuce. In contrast to the expectation based on data from other species, the *LsNDR1* gene is required for elicitation of resistance triggered by a TIR-NBS-LRR encoding gene family and is also essential for developmental processes. These findings further our understanding of the determinants of disease resistance in lettuce and provide tools for breeding programs.

PS01-088

An *Arabidopsis* Integrin-linked protein kinase 1 homologue is involved in stress response

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The plant cell wall and plasma membrane are the primary sites where environmental stimuli and the modifications they cause are perceived by cells. In animals, integrins are membrane receptors that transduce mechanochemical signals from the extracellular matrix across the plasma membrane in response to a range of stimuli. Kinase proteins named integrin-linked kinases (ILKs) bind the cytosolic domains of integrin receptors and regulate signal transduction and cytoskeletal dynamics in response to integrin activation. Several lines of evidence suggest that integrin-like signaling may exist in plants as well. For example, NDR1 has structural homology to integrin and is involved in maintaining plasma membrane-cell wall adhesion as well as activating resistance in response to R proteins (Knepper et al. 2011). We are interested in analyzing the functions of a family of plant kinases with homology to animal integrin-linked kinases. We generated homozygous T-DNA insertion lines in an ILK-like (ILL1) gene from Arabidopsis with a five-fold reduction in ILL1 transcript relative to the wild type control. Following salt stress treatments, ill1 lines demonstrated a higher percentage of cotyledon emergence and survival compared to wild type. No significant differences between lines were found following osmotic stress. Expression of ILL1 is induced in wild type following salt stress and flg22 treatments suggesting that ILL1 plays a role in general stress response. A working model of ILL1 cellular functions during plant response to stress will be presented.

PS01-089

Map-based cloning of RPS7, an additional resistance gene in *Arabidopsis thaliana* recognizing the *Pseudomonas syringae* effector AvrRps4

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The recognition of pathogen-secreted effectors is a major component of plant innate immunity and is mainly mediated by NB-LRR resistance (R) protein. However, the mechanisms by which NB-LRR proteins recognize effectors and induce downstream signaling events are poorly known. In *Arabidopsis*, the two R genes RPS4 and RRS1 are in an inverted head-to-head arrangement on chromosome 5. They are both required for recognition of AvrRps4 and PopP2, two bacterial effectors, from *Pseudomonas syringae* and Ralstonia solanacearum respectively. In accession Ws-0, the rps4-21 mutant and rps4-21/rrs1-1 double mutant fully lose PopP2 but not AvrRps4 recognition suggesting that AvrRps4 is recognized by at least another R-gene we term RPS7. Using an F2 population derived from a cross between Ws rps4-21 and RLD (which does not recognize AvrRps4 or PopP2), we mapped RPS7 ~2 cM from RPS4. In this region, we identified a pair of R genes showing similarities to RPS4-RRS1. We demonstrate that the RPS4 paralog in this pair is required for full AvrRps4 recognition in Ws-0 and, therefore, we associate this gene to RPS7. The existence of paired head-to-head R gene combinations is becoming an increasingly interesting general phenomenon.

PS01-090

Identification of novel components of the innate immunity in rice

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Plants have evolved two-branched innate immune systems called microbe associated molecular patterns (MAMPs) -triggered immunity and effector-triggered immunity to prevent the invasion of pathogenic microbes. OsCERK1 is a well-characterized MAMP receptor in rice which recognizes a kind of MAMPs, chitin. On the other hand, Pit belongs to nucleotide binding and leucine rich repeat (NLR) domain family protein that recognizes *Magnaporthe oryzea* effector, avr-Pit. In addition, *Pia* disease resistance gene locus consists of two *NLR* encoding genes, *RGA4* and *RGA5*. These genes products are necessary to recognize another *M.oryzea* effector, avr-Pia. Through *Pit* and *Pia* are known to play important roles in rice innate immunity, how these receptors regulate several immune responses is largely unknown. To further understand what is going on in cells during immune responses, we tried to identify novel components in rice innate immunity using immunoprecipitation assay. We established rice suspension cells expressing OsCERK1, Pit, an active mutant of Pit, RGA4, RGA5, or avr-Pia and detected the intact bands of these proteins using immunoblotting. We performed immunoprecipitation of some proteins. Now we are analyzing components in each protein complex using mass spectrometry. I will discuss the results in the congress.

PS01-091

Chromatin-associated regulation of plant innate immunity by the Arabidopsis PHD-finger-like protein EDM2

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Of central importance for pathogen resistance of plants are disease-resistance (*R*)-genes. They encode immune receptors that contain a nucleotide binding site (NB) and leucine-rich repeats (LRRs). Previous studies reported that NB-LRR protein and transcript levels are under tight control to allow maximal pathogen protection while avoiding spurious defense activation and detrimental autoimmunity. While post-translational mechanisms controlling R-protein functions are at least partially understood, mechanisms controlling transcription of *R*-genes are at this point largely unexplored. The nuclear localized Arabidopsis defense regulator EDM2 elevates transcript levels of the *R*-gene *RPP7* and at least two additional related *R*-genes. Both *EDM2* and *RPP7* are required for race-specific immunity of Arabidopsis against the pathogenic oomycete *Hyaloperonospora arabidopsidis*. EDM2 has typical features of chromatin-associated epigenetic regulators, such as nuclear localization signals and PHD finger-like motifs. By chromatin immunoprecipitation we found EDM2 to affect levels of dimethylated lysine 9 of histone H3. This type of epigenetic mark is known to be associated with transcriptional silencing and predominantly located to transposon loci. Consequently we found EDM2 to affect levels of this repressive mark in various transposons, including a *COPIA*-type retrotransposon located in the 1st intron of *RPP7*. At MPMI 2012, we will present new data on a chromatin-associated mechanism linking effects of EDM2 on this retrotransposon to *RPP7* expression and resistance to *H. arabidopsidis*. EDM2 serves as a paradigm for the transcriptional regulation of *R*-genes in general, providing insight on the involvement of chromatin-associated processes in the control of *R*-gene function.

PS01-092

Refining the model of R protein activation using the M flax-rust resistance protein

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Plant disease resistance (R) proteins play a vital role in defending plants against pathogenic attack. M is a flax R protein that confers resistance against strains of the flax rust fungus, *Melampsora lini*, that express and secrete the effector protein AvrM. M is a member of the most abundant structural class of R proteins, those that contain a nucleotide binding site (NBS) and a domain of leucine-rich repeats (LRR). Proteins within this class have previously been shown to possess the capacity to bind and hydrolyse ATP (Tameling et al., 2002; Tameling et al., 2006, Ueda et al., 2006 and Williams et al., 2011). Together with research into related mammalian proteins, this work has led to the formulation of a model to explain R protein activation, whereby pathogen effectors trigger a nucleotide exchange event in the R protein that leads to defence response signalling. Using *in planta Agrobacterium*-mediated transient expression and *in vitro* luciferase-based ATP quantification and hydrolysis assays, this study aimed to link the functionality of mutant M proteins with the identity of their bound nucleotide and their hydrolysis activity. We have shown that purified M protein bound with ADP can bind and hydrolyse ATP, although an autoactive M protein that preferentially binds ATP over ADP has a much higher ATP hydrolysis rate. We anticipate that these methods will allow us to uncover other residues in and around the NBS that affect the binding, nucleotide preference and hydrolysis activity of the pocket and thus the activity of the M protein.

PS01-093

Novel role for a CBL/CIPK signaling module and its targets in plant immunity

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A Ca2+ cytoplasmic increase is an early hallmark in plant innate immunity and is a necessary event for activation of downstream responses. Despite its importance, it is not yet fully understood how the information contained in Ca2+ profiles is decoded by the plant and transformed into cellular responses leading to immune responses. We identified in a large Virus Induced Gene Silencing screening (VIGS) in *N. benthamiana*, two components of a Ca2+-mediated signaling system, NbCbl10 (calcineurin B-like protein) and NbCipk6 (calcineurin B-like interacting protein kinase), as their silencing inhibited Programmed Cell Death (PCD) associated with Effector Triggered Immunity (ETI) elicited by different plant resistance genes and virus, oomycete and nematode effectors and for host susceptibility in response to two *Pseudomonas* pathogens. The tomato (*Solanum lycopersicum*) ortholog, SlCIPK6, is an active kinase and interacts in vivo with SlCBL10. Moreover, SlCIPK6 in vitro kinase activity is greatly increased in the presence of SlCBL10 and Ca2+. All together, these results demonstrate that SlCBL10/SlCIPK6 constitute a signaling module. Strategies for the identification of SlCIPK6 interacting proteins (CIPs) and ongoing characterization of candidates will be presented. Our findings reveal a novel functional role for a CBL/CIPK signaling module in plant PCD associated with immunity.

PS02-094

In vitro synthesis of the mycelial aggregate _'shiro'_ required for _'matsutake'_ mushroom production between the ectomycorrhizal fungus _Tricholoma matsutake_ and the arbuscular-mycorrhizal plant _Cedrela odorata_ regenerated from somatic embryos

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Tricholoma matsutake is an ectomycorrhizal fungus that associates with subalpine and subarctic conifers, synthesizes a rhizospheric aggregate of mycelia and soil called *'shiro'*, and produces the prized uncultivable mushroom *'matsutake'*. We raised the question if *T. matsutake* could associate with arboreal species not regarded as hosts in nature, especially those that harbor arbuscular-mycorrhizal fungi and are adaptable to the recent warm climate. *Cedrela odorata* is an ideal plant for such an analysis because it is a broad-leaved tree associated with arbuscular-mycorrhizal fungi in the tropics and is conveniently regenerated from somatic embryos, allowing axenic cultivation without natural symbionts. In the present study, we document that *T. matsutake* can interact with somatic plants of *C. odorata in vitro* and can form with the somatic plant *'shiro'* with a typical aromatic odor in a granite-based soil substrate containing the 1/4 strength of MS medium (0.5% sucrose and 0.1% glucose) at 25 °C during the 194-day incubation period. The infected plants had relatively thick epidermal tissues outside of the outer cortex, which should otherwise be developed into root hairs. Also, the mycelial sheath surrounded the outside of epidermis, and the hyphae penetrated into intracellular and intercellular spaces, forming a hyphal bundle or creating a pseudoparenchymatous organization. In the same system, *Tricholoma magnivelare*, i.e., *American matsutake*, also formed *'shiro'*. We present arguments that host-plant specificity of *T. matsutake* is not innately determined, and the somatic plant of *C. odorata* could be useful in *'matsutake'* cultivation.

PS02-095

Inconsistent role of rhizobial ACC deaminase in the root-nodule symbiosis

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The 1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme largely represented among rhizobia which converts ACC, a precursor of the plant hormone ethylene, into ammonia and α-ketobutyrate. The ACC deaminase is thought to play a crucial role in protecting the rhizobia against the effects of ethylene which interfere especially with the rhizobial proliferation and the initiation of the infection thread consequently inhibiting the formation of nodules. The beneficial action of this enzyme was demonstrated in several rhizobia such as *Mesorhizobium loti* and *Rhizobium leguminosarum* where mutations in the ACC deaminase gene showed nodulation defects (1, 2). The slow-growing rhizobial species *Bradyrhizobium japonicum* is predicted to code for an ACC deaminase (blr0241). To study the importance of this enzyme in *B. japonicum*, an insertion mutant of blr0241 was constructed and its phenotype studied. First, the ACC deaminase activity of the wild-type *B. japonicum* and of the insertion mutant was tested in free-living anoxic conditions and in soybean nodules. Although the mutant strain did not show any enzymatic activity, its ability to infect soybean, cowpea, siratro, mungbean and to fix nitrogen was not impaired. In addition, a competition assay between *B. japonicum* wild-type and the blr0241 mutant for soybean nodulation revealed that the mutant strain is not affected in its competitiveness compared with the wild-type. These unexpected results raise questions on the role, importance and mode of action of the ACC deaminase in disparate rhizobia. (1) Uchiumi et al. 2004. J. Bacteriol. 186: 2439-2448; (2) Ma et al. 2003. Appl. Env. Micro. 69: 4396-4402.

PS02-096

Nodule bacteria of mungbean (_Vigna radiata_) growing in the Central Asia

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Mungbean (*Vigna radiata*) is a well know pulse crop of Asia. It is short duration crop and can be grown twice in a year. *Rhizobium* ssp invade the root hairs of mungbean and result in the formation of nodule, where free nitrogen is fixed. The bacteria, although present in most of the soils vary in number, effectiveness in nodulation and N-fixation. Fast-growing *Rhizobium* strain was isolated from nodule of mungbean (*Vigna radiata*) growed in the experimental station of the Institue Genetica. Strains were inoculated with plants mungbean (*Vigna radiata*) and analysed in sterile condition in glass test tube experience within 30 days, in the pots experience analyzed at 60 days. In our experiments we observed formation the nodule on roots of the plants, have been selected highly effective strains. In the field experiences at the experimental station of the Institute spent experiences on the plants mungbean (*Vigna radiata*) and plants of soya. Seeds of these plants were inoculated with *Rhizobium* ssp and grown on the natural conditions. In the all plants (roots) the Soya and mungbean (*Vigna radiata*) has been formed nodule and were high productivity.

PS02-097

Identification of root-nodule bacteria isolated from desert zones of Central Asia

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From perennial plants *Onobrychis transcaucasica* and *Onobrychis corossanica* which a growing in the desert zones of Central Asia were collected nodules. After their isolation the bacteria were confirmed as rhizobia by re-nodulating their host legumes. The phenotypic characteristics utilization of carbon and nitrogen sources, tolerance to salt, heat, and antibiotic resistance there were explored. Genetic diversity of the *Rhizobium* isolates were characterized by 16s rDNA gene sequences and *nodC* gene sequences. Biodiversity were explored with (16s rDNA RFLP), REP, ERIC-BOX PCR sequences. Phylogenetic tree were constructed by RDP program. In some of the strains we found three megaplasmids, high acetylene reduction activity (ARA) and high salt stress resistance. The results obtained in this study are interesting for the molecular analysis of *Rhizobium* sp. which undergo symbiosis with *Onobrychis*, a legume plant growing in the arid zones.

PS02-098

A SNARE protein expressed in vascular tissue affects symbiotic nitrogen fixation in _Lotus japonicus_ nodules

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Rhizobial symbiotic nitrogen fixation in root nodules is regulated by the host legume genes. Fix$^-$ mutants that exhibit lower or no nitrogen-fixation activity are useful to identify host plant genes required for symbiotic nitrogen fixation. Here, we show a *Lotus japonicus* novel Fix$^-$ mutant defective of a SNARE protein. The mutant formed nodules that displayed lower nitrogen fixation activity, and the growth of the host plant was retarded. Exogenous combined nitrogen almost recovered the growth of the mutant. Numbers of nodules formed on the mutant were similar to those on the wild-type plant. However, the mutant nodules were smaller and showed early senescence. The causal gene was identified by map-based cloning, and the predicted protein was appeared to be homologous to one of SNARE proteins found in *Arabidopsis thaliana*. The responsible gene was expressed ubiquitously in shoot, roots and nodules. In roots and nodules, the transcripts were detected in vascular bundles. These results indicated that a SNARE protein expressed in vascular tissue is required for nitrogen fixation activity of rhizobia in nodules.

PS02-099

Lon protease of *Azorhizobium caulinodans* ORS571 is required for the suppression of *reb* genes expression

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Bacterial Lon protease play important roles in a variety of biological processes in addition to house keeping function. In this study, we focused on the Lon protease of *Azorhizobium caulinodans*, a kind of rhizobia, which forms nitrogen fixing nodules on the stem of *Sesbania rostrata*. The nitrogen fixation activity of an *A. caulinodans lon* mutant were not significantly different from that of wild-type strain. However, the stem nodules formed by the *lon* mutant showed little or no nitrogen fixation activity. By microscopic analyses, two kinds of host cells were observed in the stem nodules formed by the *lon* mutant. One is shrunken host cells containing high density bacteria, and the other is oval or elongated host cells containing low density or no bacteria. This phenotype is similar to a *praR* mutant highly expressing *reb* locus gene. Quantitative RT-PCR analyses revealed that *reb* locus genes were also highly expressed in the *lon* mutant. Furthermore, a mutant with deletions of *lon* and *reb* locus formed stem nodules showing higher nitrogen fixation activity than the *lon* mutant, and shrunken host cells were not observed in these stem nodules. These results suggest that Lon protease is required to suppress the expression of the *reb* locus genes and that high expression of *reb* locus genes in part causes aberrance in *A. caulinodans* - *S. rostrata* symbiosis. In addition to suppression of *reb* genes, it was found that Lon protease was involved in the regulation of exopolysaccharide production and auto-agglutination of bacterial cells.

PS02-100

Effect of external nitrogen concentration and light intensity on nodulation, nitrogen fixation and growth of cowpea (*Vigna unguiculata* L. Walp.)

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Cowpea is a legume crop able to fix atmospheric nitrogen with soil rhizobia. The objective of this study was to investigate the concentration of external nitrogen that suppresses nodulation, and the effect of light shielding on the symbiosis and cowpea growth. In a hydroponic culture experiment, nitrogen was supplied at 0, 1, 2.5, 5, 7.5, 10 mM and cowpea was inoculated with *Bradyrhizobium yuanmingense* strains TSC7, DTC8, and TTC9. The nodulation was strongly inhibited over N 7.5 mM treatment, and only a slight difference was observed between the three strains. However, the application of small amounts (2.5 mM) of nitrogen positively affects the nodulation phenotype (nodule number, nitrogen fixation) at late stage (21 DAS). To assess the effect of Photon flux density on the nodulation phenotype of cowpea associated with nitrogen-fixing rhizobia, 0, 25, 50 and 75% light intensities were set up. Defoliation was noteworthy in 25% light intensity at 10 weeks after seedling, and dry weights of pods and seeds were very high compared to the others. No difference was observed in the nodulation phenotype between the three rhizobial strains. Hence, the variation on cowpea growth may arise from the differences between red (R) and far-red (FR) light ratios of treatments. The translocation of carbohydrates might have been accelerated in the 25% intensity. However, nitrogen concentration of pods and seeds in this treatment was not so different with others. An appropriate shading would therefore, greatly improve cowpea yields, although seed protein content may not vary.

PS02-101

The rice NPC protein defines a new class of potential transporter with an essential role during AM symbiosis

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During Arbuscular Mycorrhiza (AM) symbiosis, the roots of plants and a specific group of fungi engage in a tightly regulated liaison to mutually benefit from one another. Some of the key plant elements that compose the intricate genetic network controlling AM development have been identified through the analysis of legume deficient mutants. The rice *npc* (no perception candidate) mutant is unable to properly establish AM. *NPC* encodes a previously uncharacterized potential transporter present only in prokaryotes, plants and fungi. NPC localizes to the plasma membrane and its transcript accumulates to high levels during AM. Either wild type rice or *Medicago truncatula* plants can complement the mutant phenotype *in trans*. Furthermore, amending the *npc* mutant with exudates extracted from wild type rice also results in full AM development. These findings strongly suggest the existence of a plant secreted compound(s) that acts upon the fungus or the plant to allow AM formation which is missing from the *npc* mutant. We hypothesize that NPC is a transporter involved in the secretion of one or more compounds whose function has been conserved among different plant species. To identify the chemical nature of the compound, we are comparing *npc* and wild type exudates using a metabolomic approach. In parallel, we are addressing evolutionary conservation of this communication mechanism by genetic complemenentation of the *npc* rice mutant with *NPC* orthologs from different plant species, including the early-diverging lycophyta *Selaginella moellendorffii* and the non-mycorrhizal *Arabidopsis thaliana*.

PS02-102

Evaluation of effective *Bradyrhizobium* strains from Myanmar and co-inoculation with endophytic *Streptomyces* sp.

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Soybean (*Glycine max* L.) is one of the important cash crops in Myanmar. Urea is the main source of nitrogen applied to all crops grown in Myanmar but it is very expensive and not readily available. Rhizobial inoculants can be used to substitute the nitrogenous fertilizers in food legume crops. Indigenous forty-eight root nodules bacteria (MAS1 to MAS48, where MAS means Myanmar Agriculture Service) were collected from different agro-climatic regions of Myanmar in order to evaluate their nitrogen fixing ability for soybean production. After purification, forty-three isolates gave pure colonies and were authenticated for nodule formation on host soybeans in sterilized vermiculite pots in Phytotron (25°C). Based on morphological characteristic, they were identified as *Bradyrhizobium* strains. These forty-three *Bradyrhizobium* strains were investigated in symbiosis association with Myanmar recommended soybean, Yezin-6. After inoculation, ten strains were pre-screened based on nitrogen fixation potential determined by using acetylene reduction assay method. When selected ten strains were examined in plant growth and nitrogen fixation with two Myanmar soybeans (Yezin-3 and Yezin-6), MAS23 was found being the most effective strain. The symbiotic relationship between six *Bradyrhizobium* strains (MAS23, MAS33, MAS34, MAS43, MAS48 and USDA110) and selected endophytic *Streptomyces* sp. strain (P4) were evaluated with four Myanmar soybean varieties (Yezin-3, Yezin-6, Hinthada and Shan Sein) in pot experiment by using sterilized vermiculite pots in Phytotron (25°C). It was found that dual inoculations of P4 were effectively responded in most of the soybean varieties.

PS02-103

Bradyrhizobium japonicum character predicted from genomic comparison of two strains

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The genome sequences of two *Bradyrhizobium japonicum* strain USDA6 and USDA110 have been determined. For the classification of *B. japonicum*, USDA6 has been selected as the type strain for this bacterial species. However, for various research directed towards investigating soybean symbionts, USDA110 is generally used. Comparison of the whole-genome sequences of USDA6 and USDA110 showed colinearity of major regions in the two genomes. Notably, a significantly high level of sequence conservation was detected in three regions, approximately 734 kb in total size, on each genome. The gene constitution in these indicates that they were derived from a symbiosis island. The USDA110 genome carries 14 genomic islands as the specific DNA segments inserted into tRNA genes in addition to the putative symbiosis island. Such strain-specific islands were found at ten loci in the USDA6 genome. The genes encoding enzymes in the uptake of hydrogen expressed during nitrogen fixation form a gene cluster (*hup-hyp-hox*), which have been identified on the USDA110. However, some *B. japonicum* strains, including USDA6, lack such function. Since the *hup-hyp-hox* is found inside a genomic island inserted in a *trnM* gene, it is possible that high nitrogen fixation by USDA110 might be acquired through the horizontal transfer. This genomic island is missing from the USDA6 genome. In USDA6, however, the *trnM* gene corresponding to the one targeted by the *trnM*-island in the USDA110 genome, was detected. The conservation of the *trnM* gene shows that *B. japonicum* has the potential to gain the ability to take up the hydrogen.

PS02-104

Arbuscular collapse regulates carbon release by hosts in mycorrhizal symbiosis

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Most terrestrial plant species invest substantial amounts of photosynthetically fixed carbon in arbuscular mycorrhizal (AM) symbiosis, one of the most ancient and widespread plant interaction. AM fungi are obligate root symbionts that form mutualistic associations with plants and improve their mineral nutrient uptake from the soil. Phosphate transfer to plants occurs within root cells through highly branched symbiotic fungal structures known as arbuscules. However, the mechanism by which carbon is released to the fungus remains unknown. In this study, through vital staining of fungal structures and selective lipid staining, we discovered that “lipid bursts” occur in the senescent fungal mycelia within the plant roots. Live imaging demonstrated that the appearance of lipids coincided with the collapse of arbuscular branches, suggesting that arbuscule degeneration and release of lipids from its structural constituents are associated processes. Importantly, a stunted arbuscule mutant of rice failed to produce lipids, which abolished the formation of new fungal spores. Therefore, lipid bursts are required for the fungus to complete its life cycle. This study demonstrates the existence of novel mechanism of carbon utilization by AM fungi and furthermore illustrates a cellular mechanism for underground carbon cycling that is shared by most terrestrial plant species.

PS02-105

Analysis of common symbiosis system reveals infection mechanism of arbuscular mycorrhizal fungi in _Lotus japonicus_

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Arbuscular mycorrhizal symbiosis (AMS) and root nodule symbiosis (RNS) are mutualistic plant-microbe interaction, which confers great advantages for plant growth by nutrient exchanges. RNS is known to have evolved by sharing a part of AMS system in leguminous plants. The shared symbiosis factors constitute the core of symbiosis signaling pathway, called the Common Sym Pathway (CSP). Recent RNS studies revealed various CSP factors and the signaling mechanism. These CSP factors become important cues to analyze AMS system. Calcium and calmodulin-dependent protein kinase (CCaMK) plays a crucial role for controlling CSP signaling. We found that the gain-of-function (GOF) variants of CCaMK without the regulatory domains activated both AMS and RNS signaling pathways in the absence of symbiotic partners. Furthermore, the GOF-CCaMK variant triggered formation of the pre-penetration apparatus, which is important for hyphal penetration and elongation of AM fungi in the host cell. We also found a novel AMS mutant that showed low colonization of AM fungi from RNS mutants. In this novel CSP mutant, AM fungi enter into the host root, nevertheless elongation of the hyphae was delayed or arrested, suggesting the mutant defected in the hyphal elongation mechanism. We are currently investigating detailed phenotypes during AM fungal infection and will discuss the gene function in relation to infection of the symbionts.

PS02-106

Identification of a novel _nodule inception (nin)_ mutant, _daphne_ that displays a non-nodulation but dramatically increased number of infection threads

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The symbiosis between legume and rhizobia occurs in specialized root organ called nodules. In order to establish the symbiosis, two major genetically-controlled events need to be undertaken. The first is bacterial infection with the epidermis of the root. Bacteria penetrate the root tissue from curled root hair cell and progress toward the channels called infection thread (IT). The second is the organogenesis in the root cortex. For proper establishment of symbiosis, it is essential that the two phenomena proceed synchronously in different root tissues. Although several symbiotic genes have been identified by genetic screening of non-symbiotic mutants, most of them have defects in both infection and organogenesis. The results suggest that it is experimentally difficult to examine the molecular mechanisms of the two phenomena independently. Here we isolated *daphne*, a novel non-symbiotic mutant in *Lotus japonicus*. *daphne* is completely defective in nodulation, but has increased number of ITs. By map-based cloning and inverse PCR, the reciprocal translocation was identified between chromosome II and III on *daphne* genome. Furthermore, the translocation point locates in upstream of the *NODULE INCEPTION (NIN)* gene encoding putative transcription factor that regulates both IT formation and nodule organogenesis. Allelism tests indicate that *daphne* is a new allele of *nin*. In contrast to *daphne*, it is known that IT formation never occur in other reported *nin* alleles. Thus, further analysis on *daphne* may uncover distinct regulatory mechanism of rhizobial infection and nodule organogenesis controlled by *NIN*.

PS02-107

Lotus japonicus AMP1 and *HAR1* act synergistically to regulate root architecture

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Deleterious mutations in the *L. japonicus HYPERNODULATION ABERRANT ROOT FORMATION 1* (*HAR1*) locus lead to hypernodulation and hypermycorrhization phenotypes but also restrict root length and significantly increase root branching of un-inoculated *har1-1* mutant plants. These observations indicate that *HAR1* is a central regulator of symbiotic and non-symbiotic root development in *L. japonicus*. A search for genetic suppressors of the *har1-1* phenotype lead to the identification of a root branching hypermorph, called *L. japonicus cluster root-like1* (*crl1*; so named for its superficial resemblance to genuine cluster roots). Instead of wild-type root architecture, *crl1* forms one large cluster of short rootlets with limited growth capacity. Genetic analyses have shown that the *crl1* root phenotype is determined by two independently segregating recessive mutations, *har1-1* and *Ljamp1-1*. We show that the *L. japonicas AMP1* gene encodes a predicted homologue of the *Arabidopsis* ALTERED MERISTEM PROGRAM 1 protein. As in *Arabidopsis*, the *Ljamp1-1* mutation has a pleiotropic effect on *L. japonicus* as reflected by increased cotyledon number, low fertility and short and highly branched shoots and roots. Although the *Ljamp1* single mutant root phenotype resembles *har1-1*, the *Ljamp1* mutation does not affect the symbiotic properties of *L. japonicus Gifu*, which is unlike a presumed allelic *Ljamp1* mutation in *L. japonicus* MG20 (see an accompanied abstract by T. Suzaki et al). Root architecture, however, is regulated by a synergistic action between *HAR1* and *LjAMP1* and the simultaneous impairment of these two genes results in determinate root growth.

PS02-108

The root regulator *TOO MUCH LOVE* functions in the *CLE-RS1*/*RS2*-mediated long distance control of nodulation

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The interaction of legumes with N_2-fixing bacteria collectively called rhizobia, results in the root nodule development. The number of nodules is tightly restricted through the negative feedback control by hosts. The fact that the *HAR1*-mediated control of nodule number needs the *HAR1* expression in the shoots exibits a long distance communication between the shoot and the root. However, the large part of the mechanism remains to be elucidated. Previously, we have shown that *too much love* (*tml*), a hypernodulating mutant in *Lotus japonicus*, has a defect in the negative feedback regulation and that *TML* functions in the roots downstream of *HAR1*. To better understand the mechanism by which legume plants control nodule number, we conducted molecular biological and genetic analyses using *tml* mutants. The systemic suppression of nodule formation by *CLE-RS1*/*RS2* overexpression was not observed in the *tml* mutant background. This result indicates that *TML* acts downstream of *CLE-RS1*/*RS2*. In our genetic analyses using another root-regulated hypernodulation mutant *plenty*, the *tml plenty* double mutant showed additive effects on nodule number, suggesting that *TML* and *PLENTY* act in the different genetic pathways. Together with the fine mapping of the *tml-4* and determination of the deleted regions in the large deletion alleles *tml-1*/*-2*/*-3*, the candidates for the gene responsible for the hypernodulating phenotype were narrowed down to 21 genes. Our next generation sequencing analysis identified SNPs in the region. As the gene knockdown of a candidate drastically increased the number of nodules, we concluded that it should be the causative gene.

PS02-109

Mutation of class 1 hemoglobin affects the infection of *Mesorhizobium loti* to its host plant *Lotus japonicus*

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Plant hemoglobins (Hbs) have been divided into three distinct groups: class 1, class2, and class 3 (truncated) Hbs. The class 2 Hb of leguminous plants is known as leghemoglobin in the root nodules and regulates oxygen concentrations to create suitable microaerobic environment for the nitrogenase activity of the microsymbiont rhizobia. Class 1 Hbs possess an extremely high affinity to oxygen and the various physiological functions of class 1 Hb include its role as a modulator of nitric oxide (NO) level in plants. The expression of *LjHb1*, a class 1 Hb of *Lotus japonicus*, and production of NO are induced transiently in the roots by inoculation of symbiotic *Mesorhizobium loti*. Five mutant lines of *LjHb1* were screened and inoculated with *M. loti* MAFF303099. The plant growth and nodulation of the mutant lines were inhibited compared with wild type (WT). Total numbers of infection threads and of infection drops were estimated as the infection event at 14 days after inoculation. Comparison of these numbers between WT and two mutant lines of amino acid substitution revealed that the infection event was inhibited in these mutant lines. Absorption

spectrum of the recombinant protein of the mutant LjHb1 showed that the mutation affected on the affinity to NO, suggesting that lowered NO scavenging activity of LjHb1 influenced the infection process of *M. loti-L. japonicus* symbiosis.

PS02-110

Localization of polyphosphate in arbuscular mycorrhizal fungus colonizing in *Lotus japonicus*

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Arbuscular mycorrhiza is a symbiotic association between plant roots and arbuscular mycorrhizal fungi (AMF) which promote plant growth by providing mineral nutrients, especially phosphate. The AMF form the highly branched hyphal terminus arbuscule in plant cortical cell. Arbuscules are thought to be a site of nutrient exchange between the host and the fungi. However, the mechanism of phosphate transfer from arbuscules to plant cell is poorly understood. Subcellular localizations of polyphosphate, a storage form of phosphate, and a plant phosphatase in the plant-AMF interface were observed using a transmission electron microscopy to elucidate a mechanism of phosphate transport in mycorrhiza. *Lotus japonicas* B-129 (wild type) and its RNAi line of the mycorrhiza-inducible purple acid phosphatase *LjPAP3* were inoculated with *Glomus irregulare* DAOM197198. The mycorrhizal roots were cryo-fixed, embedded in resin, and sectioned with an ultramicrotome. Polyphosphate on the sections was labeled immunocytochemicaly with the polyphosphate binding protein of *E. coli* and observed using a transmission electron microscopy. Localization of LjPAP3 was detected by immunocytochemistry with an anti-LjPAP3 antibody. Polyphosphate distributed in fungal cell walls and vacuoles of intraradical hyphae and trunks of arbuscule both in the wild type and *LjPAP3*-RNAi line of *L. japonicus*. Polyphosphate was absent in the arbuscular branches in the wild type, but was present in those in the *LjPAP3*-RNAi line. LjPAP3 was mainly localized in the periarbuscular space. These observations suggest that polyphosphate hydrolysis in arbuscules might be regulated indirectly by the plant phosphatase LjPAP3 secreted in the periarbuscular space.

PS02-111

Expression analysis of SWEET transporters in *Lotus japonicus*

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Symbiotic nitrogen fixation in legumes takes place in nodules. In infected cells of nodules, Rhizobium exists as bacteroids which are capable of reducing atmospheric N2 to NH3, whereas host plant cells provide photosynthates in forms of dicarboxylates. In this process, various transporters are involved at different membrane systems; however, little is known about the flow of carbon source from the plant cell to the rhizobia at the molecular level. In this study, we have attempted to reveal the molecular mechanism of carbon source transport to bacteroids by analyzing a putative sugar transporter expressed in nodules of *Lotus japonicus*.We focused on the gene homologs of a sugar transporter family (AtSWEET) recently identified in Arabidopsis to identify the genes involved in sugar transporter in nodules. BLAST search on genomic database of *Lotus japonicus* revealed that at least 13 homologs of SWEET exist in the genome of *Lotus japonicus*. We then performed semi-quantitative RT-PCR and found that only LjSWEET4 is highly expressed in the nodule. Real-time PCR analysis showed that the LjSWEET4 expression level in the nodule was about 10- and 3-fold higher than those of the leaves and the root tissue, respectively. It was also shown that its expression slowly increased after rhizobium infection up to 3 weeks. We also investigated the cell-type specificity in the LjSWEET4 expression using promoter::β-glucuronidase reporter gene (GUS) transformants. Membrane localization study using GFP fusion protein and elucidation of physiological functions by use of RNAi knock down transformants are underway.

PS02-112

A MATE-type transporter responsible for iron supply to nodule infection zone of *Lotus japonicus*

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Legume plants can establish symbiotic nitrogen fixation with rhizobia in root nodules, where the nutrients between host plant cells and their resident bacteria are actively exchanged. While these molecules imply nitrogen compounds, carbohydrate, and also various minerals, knowledge about the molecular basis of plant transporters that mediate those metabolite exchanges is still very limited. In this study, using the tissue-specific microarray analysis in *Lotus japonicus* nodule, we have demonstrated that a multidrug and toxic compound extrusion (MATE) protein, LjMATE1, was specifically induced in the infection zone of nodules. To characterize the transport function of LjMATE1, we conducted a biochemical analysis using a heterologous expression system with Xenopus oocyte, and found that LjMATE1 is a specific outward transporter for citrate. The physiological roles of LjMATE1 were analyzed with a gene knockdown line using RNA interference (RNAi) method, which revealed limited growth under a nitrogen deficiency condition in the presence of rhizobia compared to the control plants, whereas such a growth defect was not observed under a high nitrogen condition. We also found that Fe concentration was significantly reduced in the nodule of the RNAi line. These results suggest that LjMATE1 mediates a part of the Fe translocation from root to nodules.

PS02-113

KLAVIER is a receptor-like kinase necessary for long-distance negative regulation of nodulation mediated by CLE-RS1/2-signaling in *Lotus japonicus*

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Legume plants develop root nodules to establish the endosymbiosis with nitrogen-fixing bacteria. We have identified a novel gene, *KLAVIER* (*KLV*), encoding a leucine-rich repeat receptor-like kinase (LRR-RLK), as a responsible gene for a hypernodulating mutant of *Lotus japonicus*. Grafting between shoot and root demonstrated that hypernodulating phenotype of *klv* mutant was controlled by shoot genotype, indicating *KLV* negatively controlled the number of nodules via long-distance signaling. In leaf, *KLV* was predominantly expressed in the vascular tissues, as with another LRR-RLK gene, *HAR1*, which also regulates nodule number. Genetic analyses indicated that *KLV* and *HAR1* function in the same genetic pathway to govern the negative regulation of nodulation. *CLE-RS1* and *CLE-RS2* genes encode secretoty peptides and their expressions are upregulated due to rhizobial

infection. Overexpression of *CLE-RS1/2* in hairy root suppresses nodulation depending on *HAR1*. In *klv* mutant, the effects of overexpression of *CLE-RS1/2* on nodulation were not observed, as is the case of *har1* mutant, indicating that not only *HAR1* but also *KLV* is required for suppression of nodulation by *CLE-RS1/2*. Transient expression analysis in *Nicotiana benthamiana* indicated the physical interaction of KLV and HAR1. These results advocated a model that KLV-HAR1 receptor complex functions in long-distance negative regulation of nodulation mediated by CLE-RS1/2 signals. In this congress, we will also report the phenotypes of another allele of *klv* mutant that was recently isolated and supposed to be a null allele, and *KLV*-overexpressing plant. Reference: Miyazawa et al. Development 137: 4317-25 (2010).

PS02-114

Soybean phosphate transporter gene *GmPT7* is expressed in mycorrhizas and senescent leaves

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Soybean phosphate transporter gene *GmPT7* is expressed in arbuscular mycorrhizas (Tamura et al., 2012 Biosci. Biotechnol. Biochem. 76: 309-313). Here we analyzed *GmPT7* expression in roots at regular time intervals by RT-PCR and confirmed that the gene was induced differently from other mycorrhiza-inducible *GmPT10* and *GmPT11*. Transformed hairy roots with *GmPT7promoter-GUS* showed that GUS activity is localized in cortical cells containing mature arbuscules. Furthermore, we found high expression of *GmPT7* in senescent leaves (yellow leaves). To investigate the expression in leaves, we generated stable *GmPT7promoter-GUS* transgenic lines of soybean and detected localized GUS activity at the phloem and vein ending. In soybean, leaf senescence occurs dramatically at the end of reproduction stage. When leaves become senescent, the living components of organelles including chloroplasts are broken down and phosphorus is recycled to growing organs and seeds (Lim et al., 2007 Annu. Rev. Plant Biol. 58:115-136). Therefore, *GmPT7* may be involved in phosphate translocation from leaves to seeds.

PS02-115

Differential expression of arbuscular mycorrhiza-inducible acyltransferase and esterase genes of rice *(Oryza sativa)*

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Arbuscular mycorrhizal (AM) fungi are found in almost all soil ecosystems, more than eighty percent of plant species establishing symbiosis with them. AM fungi improve the absorption of water and mineral nutrients, such as phosphorus, nitrogen, zinc and copper, from soil to plants. In return, plants supply photosynthates to the fungi. Periarbuscular membranes in plant cortical cells accumulate organic and inorganic transporter proteins, being the main site of the nutrient exchange between AM fungi and host plants. In this work, we found that an acyltransferase *(OsAcyl)* gene and an esterase *(OsEst)* gene were highly induced during AM symbiosis of rice plants. RT-PCR analysis showed that the transcript of *OsEst* was detected slightly earlier than that of *OsAcyl* during arbuscule formation. Promoter-GUS transgenic rice plants were produced to investigate their localized expression. Both genes were expressed in cells containing arbuscules. GUS and WGA-AlexaFluor double staining indicated that *OsAcyl* and symbiotic phosphate transporter gene *(OsPT11)* were mainly expressed in cells containing young and mature arbuscules. On the other hand, *OsEst* was also expressed in plant cells nearby the penetrating intercellular hyphae at earlier developmental stages of AM symbiosis. In addition, the transcript levels of *OsAcyl* and *OsEst* were quite contrasting in a mutant rice arbuscule development of which was impaired. These results suggest that *OsEst* and *OsAcyl* genes may be involved in different functions in cortical cells. We will discuss membrane dynamics and the molecular mechanism of membrane recycling in AM symbiosis.

PS02-116

Non-redundant control of rice arbuscular mycorrhizal symbiosis by two phosphate transporters

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Phosphate (Pi) acquisition of crops via arbuscular mycorrhizal (AM) symbiosis gains increasing importance due to limited high-grade rock Pi reserves and demand for environmentally sustainable agriculture. We found that 70% of the overall Pi acquired by rice is delivered via the symbiotic route. To better understand this pathway we combined genetic, molecular and physiological approaches to determine the specific functions of two symbiosis-specific rice Pi transporters, PT11 and PT13. The PT11 lineage of proteins from mono- and dicotyledons is most closely related to Pi transporters from the ancient moss, indicating an early evolutionary origin. In contrast, PT13, arose in the Poaceae, suggesting that grasses have acquired a particular genetic redundancy to secure symbiotic Pi acquisition. Surprisingly, mutations in either PT11 or PT13 affected development of the symbiosis, demonstrating that both genes are essential for AM symbiosis. For symbiotic Pi uptake, however, only PT11 is necessary and sufficient. Consequently, our results demonstrate that mycorrhizal rice depends on the AM symbiosis to satisfy its Pi demands, which is mediated by a single functional Pi transporter, PT11.

PS02-117

Deciphering the ethylene-signaling pathway during early symbiosis in *Medicago truncatula*

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Legumes are able to establish nitrogen-fixing endosymbiosis with rhizobium bacteria. This interaction is a highly-regulated process that involves complex developmental changes in roots. Ethylene has been shown to play a key role in the early stages of the interaction, being a negative regulator of symbiotic development. In this work,

we have applied next-generation sequencing techniques (RNAseq) to analyze the changes on the transcriptome of *Medicago truncatula* roots in very early symbiotic stages (8 time points ranging from 30 min to 48 h after inoculation). To discriminate between Nod-factor and ethylene signaling, we analyzed four plant genotypes: wild type *M. truncatula* A17 Jemalong, mutants in Nod-factor perception *nfp* and *lyk3*, and a mutant in ethylene perception, *skl*. In total, we have identified almost 11,000 differentially-expressed genes, with more than 8,000 differentially regulated between wild type and *skl* samples. Among those, numerous genes are involved in ethylene perception, signal transduction and ethylene biosynthesis. Of particular interest, we have found a number of novel transcription factors of the AP2/ethylene-responsive factor (ERF) superfamily involved in very early responses (i.e., 6h) upon inoculation. We selected six candidate transcripts, based on the strength of their transcriptional response, their dependence on ethylene and/or Nod-factor signaling, and their predicted impact on transcription (i.e., positive or negative). We are currently generating promoter::GUS fusions, RNAi and overexpression constructs, along with translational fusions to GFP using hairy roots to further investigate the tissue specificity, subcellular localization and functional consequences of altered gene expression during symbiosis.

PS02-118

Rhizobial infection decides nodule identity

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Medicago truncatula forms a root symbiosis with the nitrogen fixing bacteria called rhizobia. During nodulation, rhizobia infect the root by forming infection pockets at the tip of growing root hairs after which they induce special tube-like structures called infection threads which contain the rhizobia and guide their invasion into the inner root tissues. We have identified a *M. truncatula* mutant, called *knocks but can't enter* (*kce*), that can form infection pockets but cannot form infection threads. *kce* is unable to form normal nodules but surprisingly develops short nodule-like lateral growths associated with infection foci. However the vascular bundles in those structures are centrally localised such as in lateral roots, whereas they are peripherally localised in the wild type nodules. The absence of root-tip specific markers and the expression of nodule markers suggest that these organs have a nodule identity. Interestingly, several other infection mutants that form infections blocked at a similar stage, the *M. truncatula vapyrin*, *lin*, *Lotus japonicas alb1* and the *Sinorhizobium meliloti exoY* mutant all form nodules with a central vascular bundle. This finding suggests that the abortion of rhizobial infection in the root hair curl affects the proper development of the nodule. I am testing this hypothesis by bypassing rhizobial infection, to observe whether the gain-of-function CCaMK can induce peripheral-vascular-bundle spontaneous nodules in *kce*. I am also currently testing the hypothesis that premature abortion of infection leads to changes in auxin and cytokinin balance which has direct consequences for nodule development.

PS02-119

Analysis of flavonoid secretion from the root of hydroponic culture of soybean

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Legume plants establish symbiosis with *rhizobium* to fix atmospheric nitrogen as nutrients. The first biological event of this process is the secretion of signaling molecules (e.g., flavonoids) from roots to soil, and this signal activates the transcription factor of *rhizobium* NodD, leading to the synthesis of Nod factors. As the signaling molecules in soybean, genistein, daidzein, and coumestrol have been so far identified. The transport activity of genistein has been measured with membrane vesicles prepared from soybean roots and the ATP-dependent isoflavon-specific transport has been reported (Sugiyama, 2007). In order to understand the molecular mechanisms in movement of signal molecule on the onset of symbiosis, we have characterized the root exudates in soybean. Root exudates of soybean also contain flavonoid glycosides, and the occurrence of β-glucosidase at apoplast was reported, which suggests the existence of an efflux transporter for flavonoid glycosides at plasma membrane of soybean roots. However, no transporters responsible for the secretion of flavonoids, regardless of aglycons or glycosides, have been identified thus far. In this study we have analysed flavonoid secretion during the development of soybean plants to characterize the effect of nutrient deficiency on the flavonoid secretion. Using PDA-HPLC, we have analyzed root exudates of soybean grown in hydroponic medium with various nutrient conditions. High level of secretion was observed in genistin (a genistein glucoside), daidzein, and genistein in −N medium. To identify a flavonoid transporter in soybean root, survey of transcriptionally up-regulated transporter genes in these conditions is underway.

PS02-120

Uncovering the infectome: single-cell type transcriptomic studies of *Medicago truncatula* root hairs during *Sinorhizobium meliloti* infection reveals new common symbiotic genes

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The last decade of genetic studies in model legumes has revealed that the signalling pathway of the ancient arbuscular mycorrhizal (AM) symbiosis was incorporated into the more recently evolved legume-rhizobia symbiosis. This shared pathway includes an LRR-receptor kinase and ion-channels that are required for Nod factor-induced nuclear associated calcium spiking, and a calcium calmodulin kinase (CCaMK) which is essential for triggering the transcription of the majority of gene expression changes that occur during nodulation. The fact that the recently discovered vapyrin mutant, which is impeded in intracellular infection by AM and rhizobia, has intact calcium spiking responses and requires CCaMK for its transcriptional induction suggests that elements shared between AM and nodulation are not restricted to the signalling pathway. A comparison of gene expression studies of root hairs isolated from Medicago seedlings infected by *S. meliloti* to AM-infected roots suggest that the symbioses share some hormone related responses including the induction of strigolactone biosynthesis and an enhancement of auxin signalling. In contrast, expression of genes involved in cytokinin signalling was markedly enhanced by rhizobial infection but not induced in AM interactions. As expected, the known infection-related genes, including *NIN*, *NSP1*, *RPG*, and *FLOT4* were found to be induced in root hairs during infection. Interestingly, numerous genes were found that were induced in root hairs from rhizobially infected plants, but were not expressed in nodules at any stage, indicating the enhanced sensitivity of the approach and suggesting different requirements for epidermal and cortical infection.

PS02-121

The endophyte *Epichloe festucae* requires *velvet* for a successful interaction with its host grass

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VelA, one of four conserved velvet-domain proteins, is required

for regulation of sexual development and secondary metabolism in diverse fungi. We aimed to determine the role of VelA in the mutualistic grass endophyte *Epichloe festucae*. Our results showed that VelA has only a moderate role in regulating secondary metabolism in *E. festucae* compared with other fungi. Also unlike studies in related species, there was no obvious effect on morphology, conidiation, surface hydrophobicity or the ability to grow on cell wall disrupting agents. The *velA* gene is stongly upregulated in planta versus in axenic culture. In plant interaction experiments, infection with *velA* mutants led to rapid death in 65% of seedlings, but a near wild type interaction in remaining plants, suggesting a strong effect from host plant genotype. However, experiments utilising clonal host plant lines suggest this is not a typical gene-for-gene interaction. Overall our results paint a picture of VelA regulation evolving to adapt to an endophytic lifestyle.

PS02-122

Identification of novel arbuscular mycorrhizal-specific genes regulated by gain-of-function CCaMK, a key regulator of endosymbiosis

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Common symbiosis pathway (CSP) in legumes mediates two distinct symbioses, root nodule (RN) and arbuscular mycorrhizal (AM) symbiosis. Among CSP genes, calcium calmodulin-dependent protein kinase (CCaMK) acts as a decoder of Ca^{2+} signals elicited by infection signal molecules derived from micro-symbionts. Based on detailed functional analyses with various kinds of mutated CCaMKs, we demonstrated dual regulation of CCaMK by Ca^{2+} and CaM, and differential regulation of CCaMK by CaM binding between RN and AM symbioses (1). A number of gain-of-funcion CCaMKs have been reported so far. Among them, nuclear-localizaed and deregulated CCaMK, which retains kinase domain only with T265D mutation (CCaMK1-314^{T265D}-NLS), has been shown to induce the expression of root nodule and AM-related genes strongly without infections of rhizobial bacteria or AM fungi (2). To identify novel genes required for AM symbiosis at an early stage of infection, we used dexamethasone (DEX)-inducible promoter by which expression of CCaMK1-314^{T265D}-NLS is transiently induced. Based on comparison of the gene-expression profiles in CCaMK1-314^{T265D}-NLS transgenic hairy roots of Lotus japonicus, we identified 24 candidate genes which are also expressed in response to AM fungi infection. These genes are expected to be involved in AM symbiosis. (1) Shimoda et al. Plant Cell 2012 24: 304-321; (2) Takeda et al. Plant Cell 2012 24: 810-822.

PS02-123

RNA-seq analysis of root nodules and arbuscular mycorrhiza in *Lotus japonicus* and *de novo* transcriptome assembly of *Glomus intraradices*

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Transcriptome analysis of *Lotus japonicus* was performed to identify differentially expressed genes (DEGs) in arbuscular mycorrhizal roots or root nodules compared to non-inoculated roots using next generation sequencing (NGS) technologies. Paired-end sequence reads (2x101bp) were generated by an Illumina HiSeq2000 from RNA-seq libraries. We detected 280 genes as DEG at an early stage of mycorrhizal formation (15 days after inoculation, DAI) and 6,427 genes at a late stage (27 DAI) when controlling the false discovery rate (FDR) below 0.001. In root nodule formation, 59 and 1,497 genes were differentially expressed at an early (3 DAI) and late (12 DAI) stage, respectively (FDR < 0.001). DEGs shared by the both symbioses at the late stage were 537 genes. *De novo* transcriptome assembly was conducted to gain a gene expression profile of arbuscular mycorrhizal fungus *Glomus intraradices* DAOM197198 using RNA-seq data of mycorrhizal roots. Total of 23,937 contigs (mean size = 559 bp, median size = 376 bp) were assembled by *de novo* transcriptome assembly using reads unmapped against *L. japonicus* genome sequence. 17,076 contigs (71.3%) had significant matches in a nucleotide BLAST against EST sequences in a *G. intraradices* database. The unmatched contigs were annotated with gene descriptions and Gene Ontology (GO). In all 1,204 contigs were successfully annotated. Our data suggest that expression of a large number of genes dramatically change during mycorrhization and nodulation and that transcriptome analysis of *G. intraradices* in roots is feasible by *de novo* transcriptome assembly of short reads.

PS02-124

Auxotrophic and anaplerotic amino acid metabolism in *Mesorhizobium loti*

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Current breakthroughs on signal transduction pathway for symbiotic organogenesis suggested the possibility to transfer the capability of symbiotic nitrogen fixation to major crop plants, like rice plants. To establish metabolic function in the pseudo-nodules, the profiles of plant control to symbionts should be revealed and mimic the molecular mechanism. The soil bacterium *Mesorhizobium loti* is able to induce the formation of nitrogen-fixing nodules on the root of a determinate-type legume plant, *Lotus japonicus*, and can be a model system for elucidating the molecular background of the symbiotic metabolism because genomic resources are well organized in this system. Using the signature-tagged mutagenesis (STM) technique, the functions of the up-regulated proteins in the *M. loti* bacteroids, especially of amino acid metabolisms, were surveyed. Since characteristics of transporters on symbiosome and on bacteroid membranes would be different in each legume systems, the profiles of metabolite exchange between plant cell and bacteroid might be unique in each systems. In this report we explain the data of phosphoglycerate dehydrogenase (STM5), glutamine synthetase I (STM30), ABC transporter, amino acid binding protein (STM42), argininosuccinate lyase (STM103), alanine dehydrogenase (STM95, 125) and dihydroxy-acid dehydrogenase (STM130) genes in nodules of *M. loti*/*L. japonicus*.

PS02-125

Novel arbuscular mycorrhiza-inducible phosphate transporters of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*)

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It is known that both monocots and dicots accumulate arbuscular mycorrhiza (AM)-inducible phosphate transporters (PTs) on periarbuscular membranes in infected root cortical cells, in order to absorb phosphate from AM fungi. Genes encoding subfamily I PTs of the Pht1 family, such as *OsPT11* and *LjPT4* of rice (*Oryza sativa*) and *Lotus japonicus*, are usually expressed much higher than those for subfamilies II and III PTs like *OsPT13* and *LjPT3*. Barley and wheat often show negative growth response against AM fungi, in contrast to positively responding maize and sorghum. AM-inducible PT genes of barley, *HvPT8*, and wheat, *TaPTmyc*, that encode subfamily II PTs have been reported. However, the existence of subfamily I PTs in barley or wheat remains elucidated.

Because the genome information of these crops is limited, we constructed full-length cDNA libraries from their AM roots. We also designed subfamily I-specific PCR primers, based on an alignment of DNA sequences for cereal PTs. So far, we have identified one barley and three wheat genes for subfamily I PTs. Their expression levels were apparently higher than those of *HvPT8* and *TaPTmyc*. Historically, the negative growth response was interpreted as a result of unbalance between cost (carbon supply to fungi) and benefit (phosphate transfer from fungi). Recently, however, it is often argued in relation to the interacting controls of phosphate uptake by mycorrhizal pathway through AM fungi and direct pathway via root hairs and epidermal cells. The novel genes may provide insights into the mechanism of mycorrhizal pathway in barley and wheat.

PS02-126

Study of vesicle trafficking in *Lotus japonicus* nodules

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Exocytosis and endocytosis are fundamental in plant development, homeostasis and interaction with the environment. These are highly dynamic processed that, even in otherwise static plant tissue, engage a rapid turnover of large areas of membrane surface. SNAREs (soluble N-ethyl maleimide sensitive factor attachment protein receptors) proteins drive membrane and protein targeting and delivery in eukaryotic cells. Since a specific SNARE complex is involves in membrane fusion in each vesicular transport pathway, specific organelles are marked by the presence of specific resident SNARE proteins. We have screening SNARE genes which interact with nodule development. The suppression of *Gen06* gene by the RNAi could form nodule but the most of the nodules were white in the hairly root nodule. When we infected LacZ-labeled *M. loti* , blue stained *M. loti* could see at the nodule. GFP fused gen06 protein revealed that gen06 protein was located on the plasma membrane or endosome in the *Arabidopsis* cultured cell. These data suggest that gen06 SNARE plays a vital role in the turnover of integral membrane proteins in signaling and (or) nutrition in the nodule.

PS02-127

Comparative genome analysis of *Mesorhizobium loti* strains

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Mesorhizobium loti is a member of rhizobia which can associate with legume plants (e.g. *Lotus japonicus*). *M. loti* strain NZP2037 posseses wider host range compared to other *M. loti* strains, such as MAFF303099 and R7A. In order to analyze the components that contribute to the wider host range, we have been carrying out comparative genome analysis of *M. loti* strains. As an initial step of the comparative analysis, we determined the complete sequence of the symbiotic island of NZP2037 by using conventional Sanger method, and conducted detailed comparative analysis against two strains, MAFF303099 and R7A. As a result, in the determined symbiotic island of NZP2037 with approximately 650 kbp in size, the regions of highly conserved among three strains, on which symbiosis related genes (e.g. nodulation genes, nitrogen fixation genes, conjugative transfer genes) are encoded, made up to about 30% of total size. On the other hand, the region that specific to NZP2037 was identified in the 20% portion of 3' end of the symbiotic island. The genes encoding the components of type IV secretion system (e.g. *virB1~B11*, *virA*, *virG* etc.) were conserved between NZP2037 and R7A (cf. MAFF303099 possesses type III secretion system related genes), but the genes for putative effector proteins were not conserved. In addition, two of the nodulation related genes, *nodO* and *nodU* were identified only in the symbiosis island of NZP2037. These differences could be the cause of wider host range of the strain NZP2037.

PS02-128

Does iron influence the nature of the symbiotic interaction of a fungus with its host grass?

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Siderophores are low molecular weight ferric iron chelators that are made by microorganisms to compete for and to sequester iron, an essential but potentially toxic micronutrient. *Epichloe festucae*, a fungal endosymbiont of *Lolium perenne* (perennial ryegrass), synthesises two siderophores, epichloënin and desferricrocin to harvest iron from its host grass. Previous work by our group has implicated epichloënin, and by association, iron in the maintenance of this mutualistic interaction. To explore the effect of iron availability on the grass-endophyte relationship we have created a collection of iron mutants and characterised the effects of the mutations both *in culture* and *in planta*. The iron mutants included disruptions of three components of the siderophore biosynthetic pathways and of two major iron-responsive transcription factors, including SreA that coordinates cellular responses to iron concentration changes. Iron-dependent phenotypic deviations from wild type fungal growth were observed for all fungal gene disruptions *in culture* and *in planta*. Overproduction of siderophores, relative to the wild-type fungus was detected by LCMSMS in *ΔsreA* mycelia grown in the presence of iron. Control of plant iron supply to the endophyte using hydroponic growth conditions indicated that *ΔsreA* can compete for iron with its host. Our results suggest that wild-type *E. festucae* has a tightly regulated iron management system for niche adaptation and sets limits on iron withdrawal from the host, presumably to prevent competition with its host in order to promote mutualism. Mutations that interfere with fungal iron acquisition, either by preventing or deregulating siderophore synthesis, can destabilise the fungal-plant symbiosis.

PS02-129

The expression of defense-related genes is attenuated by symbiotic signal cascades

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Plants are continuously exposed to a huge variety of microbes, including potential pathogens. To prevent the infection of these microbe, plants have evolved the sophisticated innate immune systems. In contrast, leguminous plants established the mutualistic symbiosis with soil bacteria that are collectively termed rhizobia. The mechanisms that enable the leguminous plants to accept the symbiotic microbe with preventing the invasion of pathogenic microbe are largely unclear. In the legume-rhizobia symbiosis, recognition of bacterial symbiotic signal molecules, termed Nod factors (NFs), by host plants is the key step for initiating the plant symbiotic processes. NFs are perceived by the LysM-type receptor kinases, NFR1 and NFR5. The kinase domain of NFR1 is the critical importance to activate the symbiotic cascades because the kinase domain of NFR5 lack its activity. Interestingly, the amino acid sequences of the kinase domains of NFR1 show very high similarity to that of CERK1 that is essential for recognition of chitin elicitors. Recently, we showed that only three amino acid substitutions in CERK1 kinase domain

confer the ability to drive the symbiotic signaling cascades (1). These results suggested that NFR1 have evolved from ancient chitin receptor. Here, we showed that application of NFs not only activate symbiosis genes but also activate defense-related genes through the NFR1. After 7h of NF treatment, the expression levels of defense-related genes are attenuated. We will report this genetic mechanism and discuss the role of symbiotic genes. (1) Nakagawa et al., Plant J. 65, 169-180 (2011).

PS02-130

Gene expression profiling of *Epichloe* endophytes in progenitor versus modern cereals

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Our programme aims to infect modern day wheat cultivars (*Triticum* spp.) with epichloae endophytes, sourced from wild relatives of cereals. Systemic infections of a New Zealand wheat cultivar with two genetically distinct Epichloë strains (derived from different *Elymus* spp.) were obtained, however the associations were not normal. Wheat plants infected with S18 were severely stunted and eventually died, whereas S60 infection resulted in stunted plants that were capable of the full endophyte lifecycle. We are using this system to investigate the molecular mechanisms that underlie compatibility versus incompatibility using the transcriptomic technologies SOLiD™ and Affymetrix 61k Wheat GeneChip analyses. Significant gene ontology (GO) categories were determined for differentially expressed (DE) plant and fungal genes obtained from eight comparisons derived from combinations of plant hosts (*Elymus* spp. or wheat) with endophytes S18 or S60 or endophyte free. Host genes perturbed in the artificial wheat-endophyte associations included gibberillin biosynthesis and plant defence responses suggesting these processes are involved in the symbiosis with wheat. Fungal genes that showed altered expression in wheat associations, compared to the natural host, included those involved in stress response, chromatin remodelling and cellular cell wall organisation. Peptidase/endopeptidase activities, cellular iron homeostasis and other gene ontology categories were identified as processes setting the incompatible wheat associations apart from the compatible wheat associations. Elucidation of the processes underlying compatibility will assist us in developing desired combinations of endophyte with modern day cereal hosts.

PS02-131

Synthesis and symbiosis-related gene-inducing activity of Myc-LCOs and their *N*-acyl chain-modified derivatives

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Arbuscular mycorrhizas formed between more than 80% of land plants and arbuscular mycorrhizal (AM) fungi belonging to the Glomeromycota are the most common and widespread symbiosis on our planet. The plant-AM-fungus interaction is initiated by mutual signal exchange between the two partners during preinfection stages. Host roots release strigolactones that induce hyphal branching, a host recognition response, in AM fungi. AM fungi have long been postulated to produce signal molecules called “Myc factors” (MFs) that induce the molecular and cellular responses leading to successful root colonization by AM fungi. Recently, lipochitooligosaccharides (Myc-LCOs) were identified as an MF from the germinated spore exudates of an AM fungus *Glomus intraradices* (Maillet *et al.*, 2011). Myc-LCOs were characterized as sulfated or non-sulfated tetrameric chitooligosaccharides, *N*-acylated with a C16 or C18 fatty acid moiety either saturated or having one or two unsaturations. In this study, we chemoenzymatically synthesized sulfated/non-sulfated Myc-LCOs and their *N*-acyl chain-modified derivatives, and tested their activity for symbiosis-related gene induction in *Lotus japonicus* wild-type and symbiotic mutants, *nfr1*, *nfr5* and *nfr1/nfr5* plants. Non-sulfated Myc-LCOs strongly induced the expression of the symbiosis-related genes, *SbtS*, *SbtM1* and *NIN*, in the wild type. The data will be presented on the gene expression profiles of *L. japonicus* roots upon treatment with Myc-LCOs and their *N*-acyl chain-modified derivatives in comparison to those obtained upon treatment with NF and chitin oligosaccharides.

PS02-132

Phagocytic incorporation of PCC6803 cells in *Paramacium bursaria* and RAW264.7 cells

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A variety of photosynthetic organisms can be found on the earth not only within the kingdom Plantae but also in the kingdoms Monera, Protista, Fungi and Animalia. Origins and diversities of photosynthetic organisms could be possibly attributed to the endosymbiotic theory of evolution which suggests the origin of chloroplasts to be an archetypal photosynthetic bacteria. In the last decade, we have been engaged in the study of endosymbiosis in green paramecia (*Paramecium bursaria*) in which some hundred cells of *Chlorella*-like green algae can be found within a single host cells. To date, green paramecia is the only model which allows direct observation of the very first events in the evolutionary emergence/diversification of photosymbiotic organisms. Based on this model, we have recently demonstrated that free living cells of *Synechocystis* spp. PCC6803 can be introduced into apo-symbiotic cells of *P. bursaria*. By analogy to the paramecium model, there would be three criteria for novel model hosts, namely, (1) the hosting cells must be freely cultured *in vitro*, (2) the cells must be phagocytic for allowing the experimental loading of the algal symbionts, and (3) hosting cells must tolerate the oxidative stress accompanying the photosynthetic reactions by green symbionts. Interestingly, above criteria can be satisfied by the use of cell lines of RAW264.7 murine macrophages. Here, we report on our primary attempts for introducing and maintaining the cells of PCC6803 in the RAW264.7 cells under the light condition. A very first step towards the creation of green mammalian cells was initiated.

PS02-133

Role of vitamin B6 metabolic pathway in symbiotic root nodules of *Lotus japonicus*

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Leguminous plants form nitrogen-fixing root nodules. Recently it was reported that *Mesorhizobium loti* contained the all genes of vitamin B6 <VB6> degradation pathway that was largely similar to Pathway A of VB6 degradation known in *Pseudomonas* sp. MA-1. However, It is unknown why rhizobia have such a pathway. The growth rate of *Lotus japonicus* plants treated with 0.5 or 10 μM VB6 were promoted, though that of plants treated with 100 or 1000 μM VB6 were inhibited. Plants inoculated STM strains, which have retrotransposon insertion at genes *mll6785* <pyridoxine-4-oxidase> and *mlr6806* <aspartate aminotransferase> encoding the enzyme of first and second step of VB6 degradation pathway, showed decreased growth of roots and shoots compared with those of plants inoculated wild type *M. loti* MAFF303099. Interestingly, total VB6 and free

VB6 <pyridoxine, pyridoxal, and pyridoxamine> levels in shoots of plants inoculated STM strains were largely decreased compared with those of the case of wild type *M. loti*. In root nodules formed by STM strains, expression level of bacterial VB6 degradation and biosynthetic pathway genes were decreased compared with the case of root nodules formed by *M. loti*. Moreover, expression level of VB6 degradation pathway and *de novo* pathways genes were increased in nodules formed by *M. loti* compared with those in free living *M. loti*, but not in nodules formed by STM strains. These results suggest that symbiotic nodules play an important role for VB6 metabolism of *L. japonicus* plants.

PS02-134

Development of tools for the biochemical characterization of the symbiotic receptor-like kinase DMI2

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The *Medicago truncatula DMI2* gene encodes a leucine rich-repeat receptor-like kinase that is essential for symbiosis with nitrogen-fixing rhizobia. While phenotypic analyses have provided a description for the hosts responses that are mediated by DMI2, the biochemical mechanism by which DMI2 mediates symbiotic signaling remains enigmatic. If we are to elucidate how DMI2 mediates symbiotic signal transduction, it is essential that we develop tools with which we can monitor and purify DMI2 from its native root and root nodule environment. We have generated stably-transformed *M. truncatula* lines that express a genomic *DMI2* construct that is fused to a dual affinity tag containing three copies of the hemagglutinin epitope and a single copy of the StrepII tag (*gDMI2:HAST*). We demonstrate that *gDMI2:HAST* fully complements the *dmi2-1* mutation and that transgenic plants expressing this construct behave similarly to wild-type plants. We show that the expression patterns of *gDMI2:HAST* recapitulate those of endogenous *DMI2* expression and that we can routinely detect and purify DMI2:HAST from microsomal root and nodule extracts. To facilitate DMI2 purification and characterization from root nodules, we have crossed *gDMI2:HAST* into the supernodulating *sunn* background. These tools will be a valuable resource for the *Medicago* community to dissect the biochemical function of DMI2.

PS02-135

Characterization of NO-inducing lipid A from *Mesorhizobium loti* lipopolysaccharide

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[Bachground] *Mesorhizobium loti* is a member of rhizobia and forms nitrogen-fixing symbioses with several *Lotus* species. Recently, it was reported that *M. loti* bacterial cells and their extracts induced nitric oxide (NO) in the root of *L. japonicus*. We further found that, lipopolysaccharide (LPS), a bacterial surface component, is a responsible compound for the NO induction. In this study, we characterized the chemical structure responsible for NO-inducing activity of lipid A from *M. loti* LPS. [Methods] *M. loti* MAFF303099 was grown in mannitol broth. LPS was separated by phenol-hot water extraction followed by hydrophobic interaction chromatography. LPS was partially hydrolyzed with hydrazine or aq HF to obtain O-deacylated or dephosphorylated LPS. Lipid A, a lipid anchor of LPS, was separated by weak acid hydrolysis followed by chromatographic separation. NO-induction in root was detected by fluorescence microscopy using DAF-FM diacetate. [Results] Compositional analysis showed that lipid A consisted of diamino glucose (GlcNN), galacturonic acid (GalA), and phosphate, and fatty acids which included 3-OH fatty acids and long chain 27-OH C28:0 or 27-oxo C28:0. MALDI-TOF MS and tandem MS spectra demonstrated that structure of lipid A is two GlcNN, one GalA, one phosphate and six fatty acids. NMR spectra indicated that the backbone structure is P-4-β-GlcNN(1-6) α-GlcNN(1-1)α-GalA. All lipid A fractions induced NO in the root of *L. japonicus*, but dephosphorylated one showed lower activity. These results suggests that the anionic charge may contribute to the NO induction in *L. japonicus*.

PS02-136

New regulatory peptides that that affect root nodule formation and lateral root initiation in *Medicago truncatula*

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Optimizing plant root architecture is an important agronomic goal. Root development is regulated by environmental influences and small regulatory peptides that constitute important positional signals. We have discovered a root developmental role for a peptide encoded by *MtRAR1* (*Medicago truncatula* Root Architecture Regulator 1). *MtRAR1* is part of a multigene family with a unique phylogenetic distribution being exclusive to higher plants and root knot nematodes (RKN) but not other nematodes. Environmental influences dictate expression of MtRAR genes. *MtRAR1*, for example, is up-regulated by elevated CO_2 and by nitrogen starvation and limitation. Adding the *MtRAR1* peptide to roots or overexpressing *MtRAR1* in transgenic roots leads to prolonged phenotypic changes including reduced lateral root numbers and increased root nodulation upon *Sinorhizobium* inoculation. Notably, unique periodic root swellings typified by circumferential but limited cortical, epidermal and pericycle cell divisions and elevated root hair numbers are induced which resemble RKN galls. A subset of these root swellings house arrested lateral organs likely to be developmentally arrested lateral roots but the majority show no observable signs of lateral organ formation. *Sinohizobium* inoculation leads to enhanced nodulation responses on plants overexpressing *MtRAR1* or exposed to RAR1 peptide under conditions that normally suppress nodulation. We postulate that *MtRAR1* regulates root architecture in accordance with nitrogen and carbon availability and its overexpression or overpresentation affects lateral root initiation at an early developmental point enabling sinorhizobia to highjack root development to form nodules. The expression of RKN RAR genes during RKN infection suggests a role in gall formation.

PS02-137

The use of phosphate-solubilizing rhizobacteria as biofertilizer to enhance soybean plant growth

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Plant Growth Promoting Rhizobacteria (PGPR) known for their ability to enhance plant growth in many different ways such as convert insoluble form of phosphorus to accesible form. The use of rhizobacteria as biofertilizers is one of the most promising biotechnologies to improve plant production. This study was conducted to evaluate phosphate solublizing bacteria (Cr and Crb) under *in vitro* condition and formulate them coinoculated with *Bradyrhizobium japonicum* (Bj) to determine effectiveness on soybean growth. Pikovskaya medium containing tricalcium phosphate at concentration of 0.5% was used to measure P-solubilizing ability of tested strains. Results revealed that Crb1 is the most powerful P-solublizer. Based on sequence of 16S rRNA genes, Cr and Crb isolates share higher similarity with *Bacillus* sp and *Pseudomonas* sp strains, respectively. Skim milk and molases media was used to culture isolates (Cr, Crb, Bj) prior formulation in peat as a carrier material. The combination of three strains produced 10 packages of inoculants. Each packages was tested for their viability and effectiveness on soybeans in greenhouse. The number of bacterial population after 12 months of storage was about 10^7-10^8 cells/gram of peats. Green house experiment showed that inoculants designed as F1, F2, F3, F6, F7, and F10, were significantly increased soybean plant growth. Key Words: Rhizobacteria, phosphate solubilization, 16S rRNA, plant growth, formulation, soybean.

PS02-138

Symbiotic nitrogen fixation triggers global changes in bacterial and plant sulphur metabolism

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Symbiotic Nitrogen Fixation (SNF) takes place in legume root nodules that develop after inoculation by rhizobia and involves the reduction of atmospheric nitrogen to ammonia by nitrogenase. Little is known about the molecular and biochemical mechanisms governing sulphate uptake and metabolism during SNF. In order to gain insight in the sulfur metabolism during SNF, we identified *Mesorhizobium loti* and *Lotus japonicus* genes involved in sulphate uptake, transport, reduction and assimilation. Transcript accumulation of these genes using Real Time qRT-PCR was studied in symbiotic or free-living *M. loti* strain R7A or the mutant strains *NifA* and *NifH*; both mutant strains form defective nodules with no nitrogenase activity. In contrast to *M. loti*::*nifH*, nodules harbouring *M. loti*::*nifA* strain contain no differentiated bacteroids. Furthermore, we studied the relative transcript levels of the *L. japonicus* genes in symbiotic and non-symbiotic organs of plants either non-inoculated or inoculated with *M. loti* strain R7A, *NifA* and *NifH*. Sulphate and thiols content in addition with APR activity were measured in nodules and other plant organs. Finally, sulphate flux into different sulphur pools such as cysteine, glutathione, homoglutathione and proteins was monitored by feeding external ^{35}S-sulphate to *L. japonicus* roots and nodules of plants non-inoculated or inoculated with *M. loti* strain R7A and *NifH*. Moreover, external ^{35}S-sulphate was supplied to the root system of intact plants in order to analyze the sulphate uptake and its distribution into the different plant organs. These results suggest that SNF triggers a global reprogramming of sulphur metabolism, on a whole plant level.

PS02-139

Characterization of transcription factors of *Medicago truncatula* involved in the arbuscular mycorrhizal symbiosis

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The arbuscular mycorrhizal (AM) symbiosis is a mutualistic interaction established between the hyphal network of a mycorrhizal fungus and the root system of a host plant. Here, the AM fungus (AMF) penetrates into the root cortex and develops intracellular tree-like structures, called arbuscules. These are the site of a mutualistic nutrient exchange, in which carbohydrates are provided by the plant, and mineral nutrients, predominantly phosphate, by the fungus. To sustain a functional symbiosis, the cells of colonized roots have to constantly modulate their subcellular organization, their metabolism, and their growth. Therefore, a mycorrhiza-specific transcriptional machinery is essential for the regulation of these processes. To gain a better understanding of this process, a transcription factor (TF) transcript profiling of mycorrhizal Medicago roots and non-mycorrhizal roots was carried out. Four TFs from the Ethylene Responsive Factor, the basic helix-loop-helix, and the histone-fold TF families could be identified to show a significant upregulation in mycorrhizal roots, with the promoter activity colocalizing to fungal structures. When downregulated in mycorrhizal roots by an artificial microRNA approach, the abundance of arbuscules significantly decreased in the root system. This and together with the fact that fluorescent fusion proteins of these TFs localize to the nucleus suggest their role in mycorrhiza-specific gene regulation machinery.

PS03-140

Identification of *Fusarium graminearum* secreted proteins involved in the interaction with barley and wheat

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Fusarium graminearum is a phytopathogenic fungus primarily infecting small grain cereals, including barley and wheat. Secreted enzymes play important roles in the pathogenicity of many fungi. In order to access the secretome of *F. graminearum*, the fungus was grown in liquid culture with barley or wheat flour as the sole nutrient source to mimic the host-pathogen interaction. A gel-based proteomics approach was employed to identify the proteins secreted into the culture medium. Sixty-nine unique fungal proteins were identified in 154 protein spots, including enzymes involved in the degradation of cell walls, starch and proteins. Of these proteins, 35% had not been identified in previous in planta or in vitro studies, 70% were predicted to contain signal peptides and a further 16% may be secreted in a nonclassical manner. Proteins identified in the 72 spots showing differential appearance between wheat and barley flour medium were mainly involved in fungal cell wall remodelling and the degradation of plant cell walls, starch and proteins. The in planta expression of corresponding *F. graminearum* genes was confirmed by quantitative reverse transcriptase polymerase chain reaction in barley and wheat spikelets harvested at 2-6 days after inoculation. In addition, a clear difference in the accumulation of fungal biomass and the extent of fungal-induced proteolysis of plant beta-amylase was observed in barley and wheat. The present study considerably expands the current database of *F. graminearum* secreted proteins which may be involved in Fusarium head blight.

PS03-141

Genetic diversity and PCR-based identification of potential fumonisin-producing *Fusarium verticillioides* isolates infecting corn in the Philippines

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Genetic diversity and identification of fumonisin-producing isolates of *Fusarium verticillioides* from two provinces in the Philippines were analyzed using molecular techniques. Using a Polymerase Chain Reaction (PCR)-based technique, 49 of the 54 isolates were identified as *F. verticillioides*, with an amplified product of 800 bp using VERT-1 and VERT-2 primers. Of these, VERTF-1/VERTF-2 primers detected 38 fumonisin-producing *F. verticillioides* isolates producing a single fragment of 400 bp. The other five isolates, which had previously been identified as *F. verticillioides* by TEF sequences, morphology and sexual crosses, were negative using this method. Using Universally Primed-PCR (UP-PCR) markers for *F. verticillioides*, no grouping was observed based on geographical origin and species, but intermediate (53.8%) to high (99.6%) bootstrap values and high genotypic diversity (H=0.99) were generated, suggesting that all isolates clearly belonged to *F. verticilliodes*. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis with Jaccard's coefficient showed that similarities among *F. verticillioides* isolates were intermediate at 71% similarity level.

PS03-142

Transient and multivariate system for transformation of a fungal plant pathogen, *Rosellinia necatrix*, using autonomously replicating vectors

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Rosellinia necatrix is a fungus that infects a wide range of host plants and ruins a variety of commercially important crops. DNA fragments can be introduced into *R. necatrix* using conventional protoplast-PEG transformation and genome-integrating vectors; however, the transformation efficiency with this strategy is quite low. Therefore, to establish a more effective transformation system for studies of *R. necatrix*, an autonomously replicating vector was constructed using AMA1 sequences derived from *Aspergillus nidulans*, which is distantly related to *R. necatrix*. Use of this AMA1 sequence-containing vector increased the transformation efficiency in *R. necatrix*, and the vector was maintained as a plasmid in the transformants. Transient and multivariate functional analyses in *R. necatrix* were performed using co-transformation of multiple pAMA-H vectors, each of which carried either an expression cassette for eGFP, mOrange2, or a geneticin resistance gene. Furthermore, fluorescent proteins expressed from the autonomously replicating vectors were dispersed throughout fungal colonies even though the vectors themselves were restricted to the center of each colony. This intriguing phenomenon indicated that gene products could move from the center to the margin in a colony of the filamentous fungi via a cell-to-cell transport system. RNAi-mediated gene silencing was also performed successfully by introducing a pAMA-H vector carrying sequences for dsRNA production into fungal cells. However, the effect of RNAi was not equally distributed throughout the colony, suggesting that the RNAi signal may not be transduced systemically via a cell-to-cell transport system in *R. necatrix* colonies.

PS03-143

Arabidopsis GNOM ARF-GEF and barley ARFA1b/1c GTPase link multivesicular bodies to syntaxin-regulated penetration resistance

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Basal defence against powdery mildew fungi is manifested as penetration resistance in the outer cell wall of epidermal cells, where a callose-containing papilla is formed at the site of fungal penetration. The orthologous plasma membrane syntaxins, PEN1 and ROR2, of Arabidopsis and barley have previously be implicated in penetration resistance. These syntaxins accumulate at the site of attack as they become embedded in the papilla, and we consider these as markers for exosomes secreted from multivesicular bodies during the build-up of the cell wall apposition. Syntaxins belong to the SNARE proteins involved in vesicle fusion. Meanwhile, vesicle budding is regulated by AFR GTPases, which in turn are activated by ARF guanine nucleotide exchange factors (ARF-GEFs). We found that BFA, that targets certain ARF-GEF, inhibits penetration resistance in Arabidopsis in a PEN1-dependent manner. Furthermore, BFA inhibits deposition of callose and GFP-PEN1-labelled exosomes in papillae. By introducing different mutant versions of GNOM into our plant material, we where able to demonstrate that this BFA-sensitive ARF-GEF is involved in penetration resistance, and we confirmed that PEN1 and GNOM function on the same pathway. In a parallel study in barley, we used transient single cell RNAi-based gene silencing to screen for ARF GTPases involved in penetration resistance. Thereby, we identified HvARFA1b/1c to be essential for this type of basal defence. Subsequent analyses using over-expression of dominant-negative versions of this HvARFA1b/1c demonstrated that it is important for ROR2-regulated penetration resistance and deposition of callose and YFP-ROR2 in papillae. Confocal studies associated HvARFA1b/1c-GFP with multivesicular bodies.

PS03-144

Isolation of plant and powdery mildew components defining and controlling formation of the extrahaustorial membrane

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Powdery mildew diseases are caused by obligate biotrophic Ascomycete fungi of the order Erysiphales that completely adapted their molecular machinery to modulate host defences and cellular trafficking. For nutrient and effector exchange the host cell is tricked to allow a specialized intracellular feeding structure, the haustorium to be formed. The membrane surrounding the haustorium, the extrahaustorial membrane (EHM), is plant derived. However, the subcellular origin, molecular mechanisms and the fungal effectors redirecting the plant molecular machinery to form the EHM are unknown. The model system used is the interaction between barley (*Hordeum vulgare*) and the powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*). We use three approaches to study EHM formation. Firstly, by particle bombardment, fluorescently labelled organelle marker constructs and sequences for transient induced gene silencing are co-expressed in barley epidermal cells. We will specifically interfere with or silence components of the vesicle trafficking machinery. Subsequently, pathogen ingress and marker localization can be followed at single cell level using fluorescence microscopy. Secondly, the EHM proteome will be analysed by MS-MS using an optimized haustoria isolation protocol. Here special care is taken to maintain the EHM, in order to identify plant components defining the identity and controlling the formation of this membrane. Finally, a *Bgh* fungal cDNA library will be stably expressed in Arabidopsis after *en masse* transformation to identify fungal components that modulate plant vesicle trafficking and secretion. We foresee a drastic effector mediated modification of the plant secretory pathway.

PS03-145

A pH-responsive transcriptional factor is involved in the entry mode selection of *Colletotrichum orbiculare* at wounded sites of Arabidopsis leaves

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Colletotrichum orbiculare (*Co*) is the causal agent of cucumber anthracnose disease. To infect host plants, *Co* forms melanized appressoria that enable the fungus to invade the plants. However, we have recently reported that *Co* exhibits hyphal tip-based entry (HTE), uncoupled with formation of melanized appressoria, at wounded sites of nonhost *Arabidopsis* leaves. Here we show the involvement of a pH-responsive factor in the entry-mode switching to HTE in *Co*. The transudate collected from wounded sites of *Arabidopsis* leaves induces HTE-like morphogenesis of *Co* on an artificial hydrophobic surface. Interestingly, its activity severely decreased when the transudate pH was shifted, implying the link of extracellular pH condition to HTE. Consistently, we found that appressorium development of *Co* on the hydrophobic surface was suppressed when ambient pH was shifted to alkaline condition. PacC is known as a pH-responsive transcriptional factor in several filamentous fungi. To assess the potential involvement of PacC in the switching to HTE, we identified the *PacC* homolog of *Co* (*CoPacC*) and generated *CopacC* null mutants. As a result, the transudate induced HTE-like morphogenesis of *Co* in the CoPacC-dependent manner. Furthermore, at wounded sites of *Arabidopsis* leaves, the ratio of melanized appressorium formation in the *CopacC* mutants significantly increased in comparison with that of the wild type, indicating the roles of CoPacC for HTE at plant wounded sites. Thus, these results strongly suggest that the *Co* switches to HTE via CoPacC-dependent regulation in response to environmental changes.

PS03-146

Tracking of esca causal agents, *Phaemoniella chlamydospora* and *Phaeoacremonium aleophilum*, in young vine plants

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Grapevines are sensitive to a wide range of fungal pathogens. Among them the progression of grapevine trunk diseases (GTD) represents a real threat for viticulture. Esca, a particular GTD, is caused by tracheomycosis induced by *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. Here we have tracked microbe-plant interactions in vine wood via different approaches. Firstly we developed a 3-plex real-time quantitative PCR method to detect and quantify these microorganisms in grapevine wood samples from experimentally infected vines as well as young vines from the nursery. We have shown with inoculated cuttings that both fungi colonized the wood well, even in the absence of visual phenotype for *Phaeoacremonium aleophilum*. The analysis of samples of young vines from the nursery showed that most of the positive cases were found at the base of the plants rather than at the graft point. We reproduced similar quantitative results in confined conditions for *Phaeomoniella chlamydospora* using low inoculum density in soil (100 conidia per gram). Secondly we analyzed during early infection events how the wood develops defense mechanisms at molecular level. Using RT-qPCR techniques on RNA extracted from wood after treatment with different pathogens, expressions of defense genes within hours post-inoculation will be presented (i.e. STS, Chitinases, Lox, PAL). These results would reveal how fast woody tissues are able to detect and react to the presence of these fungi. Finally we have begun development of histology analysis tools to characterize fungi localization (FISH methodologies) and plant tissue response to the attack of esca associated fungi.

PS03-147

A possible alternative target of Roxithromycin in fungi

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Magnaporthe oryzae is the causal agent of rice-blast disease. It is considered to be important to reveal its infection mechanism to host-plant. *M. oryzae* enters its host plant using a specialized infection structure known as an appressorium. The developmental stage of appressorium is sensitive to various chemical inhibitors, because large numbers of genes are involved in cellular differentiation. Since appressorium formation by *M. oryzae* can be observed on artificial surfaces, it can be a useful tool to search new activity of various chemicals. We searched novel molecular targets of authentic chemicals by using this fungus. The Roxithromycin (RXM), which was originally active against prokaryote, inhibited appressorium formation of *M. oryzae*. RXM has beneficial side effects such as anti-inflammatory activities were reported and actually applied to human. However, the mechanisms underlying these effects are unclear. These results suggest that there are alternative targets in broad eukaryotic organisms and it is interesting to identify the molecular target of the secondary effect on human using *M. oryzae*. We performed phage display to search novel molecular target(s) of the antibiotic. Candidate gene 32-11 mutants, expression of 32-11 gene was lower than wild type during developing infection structure, were less affected by RXM, although germinate and formation of appressoria were normal. Over expression of 32-11 caused no effect to RXM activity, germination or appressorium formation compare to the wild type. These results possibly suggest that the complex of 32-11 product and RXM affects another molecule which plays an important role in appressorium formation at *M. oryzae*.

PS03-148

A Plant-microbe interaction between strawberry cultivar Ecchiesu-138 and the causal *Alternaria* pathogen is homologous with that between cultivar Morioka-16 and the strawberry pathotype of *A. alternata*

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The Alternaria black spot of strawberry is caused by the strawberry pathotype of *Alternaria alternata*, which produces host-specific toxins, called AF-toxins, and affects only one Japanese strawberry cultivar, Morioka-16. Under laboratory conditions the strawberry pathotype is also pathogenic to a narrow range of the Japanese pear cultivars susceptible to the Japanese pear pathotype of *A. alternata*. In 2009, the occurrence of black spot on the recently bred strawberry cultivar Ecchiesu-138 (HS-138) and the taxonomic examination of the causal *Alternaria* pathogen were reported by Misawa *et al*. Following the report, the plant-microbe interaction between HS-138 and the causal pathogen was compared with that between Morioka-16 and the strawberry pathotype of *A. alternata*. The pathogenic isolate E11 from HS-138 was confirmed to have pathogenicity not only to Morioka-16 strawberry but also to Nijisseiki, which is a Japanese pear typically susceptible to both the strawberry and the Japanese pear pathotypes of *A. alternata*. Isolate NAF8 of the strawberry pathotype *A. alternata* showed pathogenicity to HS-138. Production of AF-toxins by the isolate E11 was found by chromatographic analysis and bioassay on the leaves of Morioka-16 and Nijisseiki. On the other hand, HS-138 plants were affected by isolate NAF8 by spore inoculation and by AF-toxin I to the same degree as Morioka-16. These results suggest that HS-138 plants are susceptible to the strawberry pathotype because of their sensitivity to host-specific AF-toxin. A comparison of CD chromosomes which have gene clusters for AF-toxin biosynthesis among the isolates will be discussed in this report.

PS03-149

Switching between pathogenicity and saprophytic phase in *Heterobasidion annosum*

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Initial colonization of a fungal phytopathogen to host dead tissue involves expression of a group of pathogenicity factors, cell wall degrading enzymes (CWDEs) which provide nutrients for its growth and ability to infect healthy tissue. This action bounds the pathogenicity phase to saprophytic phase. The transition of these two phases is effected by the levels of cAMP and glucose. Declining cAMP levels and glucose becomes available can cause repression of hydrolytic enzyme synthesis. Low glucose (high cAMP) levels induce CWDEs synthesis and tissue necrosis. in vivo results showed that high level of glucose can induce the pathogenicity of *Heterobasidion annosum* germinating spore. But when constant glucose was supplied to the mycelium, the pathogenicity phase was much delay. To understand the mechanism behind this process (Switching between pathogenicity phase and saprophytic phase), we have developed a transformation system for *Heterobasidion annosum* and showed the potential ability to be a useful genetic tool. Further identification of the key genes involved will be presented.

PS03-150

Global expression profiling of transcription factor genes provides new insights on pathogenicity and stress responses in the rice blast fungus

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Most efforts to understand the molecular mechanisms of pathogenicity in fungi have focused on studying the role of individual genes, and little is known about how the expression of pathogenicity genes is regulated and coordinated in a whole genome scale. Rapid increase in fungal genome sequencing and availability of efficient tools for global gene expression analysis help address this deficiency. Here we analyzed expression patterns of 206 genes encoding transcription factors (TFs) in the rice blast fungus under 32 conditions including infection-related developments and various abiotic stresses using qRT-PCR. Resulting data, which is publicly available via an online platform, helped understand the regulation and potential interactions of these TFs in controlling responses to a diverse array of stimuli. High level of differential expression was observed during fungal developmental and abiotic stresses conditions. More than 50% of TF genes were up-regulated during conidiation. Both insertion and deletion mutants of corresponding conidiation-specific TFs showed defects in conidiation, suggesting the accuracy of the expression data to predict their function. Large overlaps of expression patterns were found between *in planta* and oxidative stress-responsive TFs. Phenotype analysis of corresponding T-DNA insertion mutants showed not only sensitive to oxidative stresses but also failed to infection in the host. The proposed regulatory network via the TFs analyzed in this study will facilitate studies on the function and potential interactions of individual TFs in regulating pathogenicity in *M. oryzae* and will also serve as a reference in studying the mechanism underpinning evolutionary fine-tuning of transcriptional regulation in fungi.

PS03-151

Investigating the role of deduced polarity establishment factors, CoCDC42 and CoBEM1, in infectious morphogenesis of *Colletotrichum orbiculare*

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Colletotrichum orbiculare is the causal agent of anthracnose of cucumber. The infection process involves a series of cellular differentiation; conidia of this fungus germinate, elongate the germ tubes, and the tips of germ tubes then produce darkly melanized appressoria which develop a penetration peg to penetrate and colonize the host plant tissues. Previous investigation in our laboratory revealed that *CoKEL2*, a homolog of *Schizosaccharomyces pombe TEA1* encoding a cell-end marker protein for cell polarity, was required for appressorium development in *C. orbiculare*. However, the role of polarity establishment factors in this process has poorly understood. Cdc42, a Rho-type small GTPase, is known to be a central polarity-establishment factor in a variety of eukaryotic organisms that organizes various processes necessary for polarization, such as actin organization and membrane trafficking. Bem1 is known to be the scaffold protein that is required for proper Cdc42p activation in *Saccharomyces cerevisiae*. In this study, deletion analysis revealed that *CoCDC42* and *CoBEM1*, homologs of *S. cerevisiae CDC42* and *BEM1*, respectively, are both required for the full virulence of *C. orbiculare*. The *cocdc42* mutants exhibited pleiotropic defects including delayed growth, decreased conidiation, abnormally shaped conidia, and altered germination patterns, but still formed appressoria that retain the ability to penetrate the host plants. On the other hand, the *cobem1* mutants exhibited phenotypic defects that partially overlap with those observed in *cocdc42* deletion mutants. Further experiments are being carried out to investigate the precise function of CoCDC42 and CoBEM1 during infection-related morphogenesis of *C. orbiculare*.

PS03-152

***MoERR1* encoding an ER retention protein receptor is required for asexual development and pathogenicity in the rice blast fungus**

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Endoplasmic reticulum (ER) is known as a key organelle for post translation process and secretion in eukaryotes. *Magnaporthe oryzae* has two *ERD2* othologs of *Saccharomyces cerevisiae*, which function as protein retaining receptors in ER and Golgi membrane. *MoERR1*, one of *ERD2* orthologs, was identified from the transformant obtained from *Agrobacterium tumefaciens*-mediated transformation library as a pathogenicity defective mutant. Additionally, we did targeted gene disruption of both *MoERR1* and *MoERR2*. GFP tagging of MoERR1 and MoERR2 showed that MoERRs were localized to ER membrane. *moerr1* mutant showed defects in expression of ER-related genes under ER stress conditions. *moerr1* mutant formed small and round-shaped conidia and was also defective in mycelial growth and conidiation. *moerr1* mutant was unable to penetrate the plant surface due to defect on turgor generation in mature appressoria. However, *moerr1* mutant incited blast lesions when wound-inoculated, indicating that *moerr1* mutant has the ability to grow in planta. Unlike *moerr1* mutant, *moerr2* mutant showed no significant phenotypes compared to the

wild type. These results demonstrate that *MoERR1*-mediated ER functions are required for asexual development and appressorium-mediated plant infection in the rice blast fungus.

PS03-153

Cellular dynamics of *Magnaporthe oryzae* during infection process both on hydrophobic (leaf) and hydrophilic (root) surfaces

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Magnaporthe oryzae is known as a causal agent of blast disease in cultivated gramineous crops. This fungus takes various differentiation patterns depending on the environmental condition. On the leaf surfaces, the spores elongate germ tubes and differentiate appressoria. However, on the root surfaces, immature appressoria known as hyphopodia were differentiated at the tip of the germ tubes. Recent molecular biological studies suggested that autophagy induction and metabolism of storage substance in the spores were important for functional appressoria. Although cytological analyses were examined, these studies were targeted to not spore but hyphae because of technical difficulty and never captured autophagosome that was typical features of macroautophagy. Cytological report of hyphopodia differentiation was also rare. In this study, we evaluated cellular dynamics by transmission electron microscopy (TEM) during infection process both on hydrophobic (leaf) and hydrophilic (root) surfaces, especially focused on autophagy machinery. Furthermore, we examined the correlation between the autophagy and metabolism of the storage substance in the spores. TEM observation in the spores producing appressoria at 12 hours post inoculation (hpi) revealed that many autophagosome-like vesicles were accumulated at the adjacent regions of enlarged vacuoles. At 24 hpi, most of the organella in the spores disappeared. In contrast, on the root surfaces, such vesicles had never observed during the infection process. Moreover, we found that transfer of storage substance in spores hardly occurred toward the hyphopodia. These results suggested that autophagic machinery and relevant metabolic pathways is important switching determining organ specific pathogenicity in *M. oryzae*.

PS03-154

A putative lipid phosphate phosphatase is required for defense responses in *Arabidopsis thaliana* to adapted and non-adapted pathogens

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A mutation in the *Leptosphaeria maculans susceptible1* (*LMS1*) gene encoding a putative plastid-localized phosphatidic acid phosphatase-like protein is involved in glycerolipid metabolism that induces premature senescence and impairs resistance to diverse fungal and oomycete pathogens in *Arabidopsis thaliana*. Increased accumulation of oleyl-acyl carrier protein, the starting substrate of glycerolipid metabolism, was observed for *lms1* under the no biotic stress condition, while enhanced activation of the prokaryotic pathway-dependent lipid synthesis was suggested for *lms1* during pathogen challenge. The *acyltransferase* (*act1*) mutation retarding the first committed step of the prokaryotic pathway or the *sulfoquinovosyldiacylglycerol* (*sqd2*) mutation that hampers SQDG synthesis suppressed the *lms1* disease susceptibility and the accelerated senescence phenotype. The *salicylic acid* (SA) *induction deficient* (*sid2*) mutation blocking pathogen-responsive SA synthesis also suppressed the *lms1* disease susceptibility in the *sid2 lms1*double mutant. Application of SA restored the *lms1* phenotype in *sid2 lms1*, highlighting the role of SA signaling in the *lms1*-conditioned disease susceptibility. SA, however, did not affect the phenotype of *act1 lms1* and *sqd2 lms1*, indicating that SA signaling alone does not fully account for the *lms1* phenotype. We propose that the *lms1*mutation involves SQDG upon pathogen attack, resulting in over-activation of SA signaling, which collaborates with a yet unidentified signal to trigger the *lms1* disease susceptibility.

PS03-155

Putative components of a protein complex for processing of ACR-toxin Sensitivity gene (*ACRS*) mRNA

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Alternaria alternata rough lemon pathotype (*A. alternata* RLP) causes Alternaria leaf spot disease of rough lemon (*Citrus jambhiri* Lush.). *A. alternata* RLP produces a polyketide host-selective toxin, called ACR-toxin. The site of action of ACR-toxin has been established to be the mitochondrion. The host-selectivity of ACR-toxin is extremely high and only rough lemon among commercially available citrus cultivars is sensitive to this toxin and therefore susceptible to *A. alternata* RLP. We identified a rough lemon mitochondrial DNA sequence, designated *ACRS* (ACR-toxin Sensitivity). Expression of this gene confers toxin sensitivity to *Escherichia coli*. *ACRS* is located in the group II intron of citrus mitochondrial tRNA-Ala. Sensitivity to ACR-toxin is due to differential post-transcriptional processing of *ACRS*mRNA. *ACRS* is translated into a SDS-resistant oligomeric protein in rough lemon mitochondria but not in the toxin-insensitive mitochondria. We identified *ACRS*mRNA-binding 30kDa protein (AmBP30) from toxin-insensitive citrus mitochondria. However, AmBP30 protein alone was not sufficient to make a processing of *ACRS*mRNA. Multiple steps by a protein complex with subunit proteins with different functions are usually required for RNA processing. To isolate the proteins consisting AmBP30 complex, toxin-insensitive citrus cDNA library was screened by yeast two hybrid (Y2H) using AmBP30 protein as the bait. Immunoprecipitation (IP) using anti-polyclonal antibodies for AmBP30 and Y2H-identified proteins following TOF-MS analysis identified two component subunit proteins interacting with AmBP30.

PS03-156

Dissection of genes involved in trichothecene biosynthesis and virulence in *Fusarium graminearum*

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Fusarium graminearum species cause Fusarium head blight (FHB) of wheat and other small grain cereals worldwide, producing various types of trichothecene mycotoxins that are toxic to human, domestic animals, plants and yeast. Trichothecene mycotoxins including deoxynivalenol, nivalenol and their acetylated derivatives are the principal mycotoxins produced by *F. graminearum* and are considered the predominant mycotoxin contaminants in food/feed stuffs in China and many other countries. Paired isogenic isolates differing in single-specific genes can be generated and used for the comprehensive investigations of gene functions through combined genetic and molecular characterization, and

metabolic analysis by NMR spectroscopy. This is essential for the development of novel strategies for effectively controlling FHB and the associated mycotoxins in food/feed chains. Metabolic analysis enabled identification of a limited number of metabolites with assigned chemical structures, in contrast to a huge magnitude of gene transcripts derived from transcriptome analysis, most of which are in general not easily assigned for their nature and roles. Many metabolic changes due to loss of a single gene, such as Tri5-deletion, in a FHB pathogen identified by the combined metabolite and transcript analyses display distinctly altered patterns of carbon and nitrogen metabolisms, as both primary and secondary metabolic compounds, as well as transportation regulations and nucleic acid biosynthesis, in addition to its defined function. Therefore, the combination of genetic, molecular and metabolic analyses would be a powerful way to reveal the systemic molecular and metabolic roles of individual genes in a mycotoxin-producing fungal organism.

PS03-157

Functional analysis of germ tube expressing cDNA library of *Magnaporthe oryzae*

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The filamentous fungus *Magnaporthe oryzae* causes rice blast, the most serious disease that affects global rice production. On the surface of host plant, a specialized infection structure called appressorium is formed on tip of germ tube. Induction of the development of appressorium requires several external stimulants and a complete cycle of cell division. Although many studies have revealed some of process of appressorium formation in *M. oryzae*, the complete mechanism is still obscure. We selected B51 gene from germ tube expressing cDNA library and made B51 gene disruptants. The cDNA library mainly contains the genes that express in the period of germ tube development and/or appressorium formation. B51 gene is presumed to have forkhead associated (FHA) domain, which is contained in many proteins that are involved in DNA repair and cell cycle. In our previous study, B51 gene disruptants demonstrated pleiotropic effects. Although *Neurospora crassa* knock-out mutants of rcaA, which share sequence similarities with B51 gene, showed similar phenotypes to B51 disruptants, rcaA did not seem to contain FHA domain. Toward further study of the function of B51 gene, we induced a plasmid carrying an rcaA (pNB51) into B51 gene disruptants. Consequently, pNB51 was able to partially complement phenotypes of B51 gene disruptants. This result suggested that rcaA has at least partial similar functions of B51 gene in *N. crassa*.

PS03-158

Transcriptional regulatory circuits necessary for appressorium-mediate plant infection by *M. oryzae*

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To cause rice blast disease, the fungus *Magnaporthe oryzae* elaborates a specialized infection structure called an appressorium, which use enormous turgor to rupture the tough outer cuticle of a rice leaf and allow the fungus to invade living plant tissue. The *M. oryzae* Pmk1 MAP kinase pathway is essential for pathogenicity and regulates appressorium morphogenesis. The Pmk1 MAP kinase pathway leads to activation of physiological changes, such as lipid body mobilization and cellular differentiation essential for plant infection. The *M. oryzae pmk1* null mutant forms long undifferentiated germ tubes but does not elaborate appressoria and is consequently non-pathogenic. Moreover the *M. oryzae* Mst12 transcription factor, which is phosphorylated by Pmk1, regulates penetration peg emergence and *mst12* mutants are consequently unable to penetrate the plant. We have recently carried out genome-wide comparative transcriptional profiling analysis for both null mutants using RNA-seq and HiSeq 2000 sequencing. A comparative transcriptional profile analysis has been completed in both Pmk1 and Mst12 mutants and in a range of novel mutants affected in appressorium function. In this way, we aim to define the transcriptional signature associated with appressorium development in the rice blast fungus and define the regulatory circuits necessary for appressorium-mediated plant infection by plant pathogenic fungi.

PS03-159

Transcriptome analysis of six wheat leaf rust races

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Wheat leaf rust, caused by the basidiomycete *Puccinia triticina*, can cause yield losses up to 20% in wheat producing regions. During infection, the fungus forms an extracellular feeding structure called the haustoria. Proteins secreted from the haustoria enter the plant cell and effect changes in plant transcription, metabolism and defense. Race structure in *P. triticina* is defined by infection type on wheat lines containing different resistance genes. In this experiment, RNA was extracted from wheat leaves infected with six different rust races at six days post inoculation. Illumina Solexa sequencing reads were assembled using Inchworm. To separate sequences by species of origin, contigs were BLAST aligned to either a wheat EST database or a *P. triticina* reference genome sequence. A total of 222,571 rust contigs were assembled from a total of 165 million reads, with an average contig length of 744 bases. Translated secreted protein sequences were examined for the presence of SNPs resulting in amino acid changes and temporal expression profiles were developed for the corresponding genes.

PS03-160

Transcriptional factor(s) and the regulatory region on the 5'-upstream of the *CBP1* gene specifically expressed during appressorium differentiation of *Magnaporthe oryzae*

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The rice blast fungus, differentiates a specialized infection structure called an appressorium, which is essential to penetrate into the host plant. From our differential cDNA library including ESTs strongly expressed in appressorium formation by subtracting the cDNA in vegetative mycelia, *CBP1* (Chitin Binding Protein 1) gene was found to be the gene which specifically expresses at early stage of the appressorium differentiation. The *CBP1* 5'-upstream region was analyzed using the *eGFP* reporter gene. And the region around -854 to -696 bp of the 5'-upstream, CUR159, was assigned to be important to regulate the expression. Probably *CBP1* expression is repressed in vegetative growth by a transcriptional factor (TF). Here, the TF(s) of the *CBP1* gene was searched from the nuclear fraction of vegetative mycelia by using an electrophoretic mobility shift assay (EMSA). The candidate TFs to shift the CUR159U up in EMSA were fractionated by heparin affinity chromatography from the nuclear fraction, but still contaminated by many other proteins. When the CUR159 was divided into smaller parts, the region from -854 to -806 bp and -829 to -782 bp had the shift-up ability in EMSA. In the overlapping region, some known motifs to bind TFs were found. As the motifs were substituted to the nonsense sequences, the region from -832 to -817 bp, CUR16TF, was found to be the key region. Now using streptavidin magnetic beads and

the biotin-labeled DNA probe including CUR16TF, the proteins to bind specifically with the region are purified and identified by LC-MS/MS after electrophoretic analyses.

PS03-161

Transcriptional changes mediated by chitosan in *Colletotrichum gloeosporioides*

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Anthracnose caused by *Colletotrichum gloeosporioides Penz* is considered the most important postharvest disease of tropical and subtropical regions of the world, being the main phytopathological agent of papaya fruit postharvest worldwide. In Mexico, this disease is found in all regions, causing losses that range from 15 to 50%. Currently, fungicides are used to control this disease, which can be harmful and expensive. On the other hand, chitosan has been studied as a potential antifungal agent and could be used to control plant diseases. The effects of chitosan in the fungus *Colletotrichum gloeosporioides* grown on PDA, PDA-chitosan, papaya and papaya-chitosan were measured by molecular responses, through changes in gene expression by differential display technique; the differential fragments obtained were then sequenced and compared with the NCBI database. The MIC of chitosan was 1600 ppm which only allowed 42.3% fungal growth compared with the control group. After this test, we evaluated this chitosan concentration in papaya media and obtained an inhibition of 29.4%. We found 21 differential fragments, sequencing and subsequent alignment of the DNA differential fragments showed 4 proteins: hypothetical protein *Verticillium alboatrum* , Region ATPase of topoisomerase II of *Saccharomyces cerevisiae* , Protein tyrosine-serine phosphatase of *Nacamureya multipartite* and the transcriptional Mediator of RNA polymerase II. The chitosan is effective as antifungal agent against *C. gloeosporioides* because it represses key factors for replication and transcription process of this fungus, which could have effects on the regulation of metabolism itself.

PS03-162

Genome rearrangements abolishing the ability of *Rosellinia necatrix megabirnavirus1* to confer hypovirulence to the white root rot fungus

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Rosellinia necatrix megabirnavirus1 (RnMBV1), a novel bipartite double-stranded RNA mycovirus isolated from the ascomycete *R. necatrix*, is a destructive pathogen of perennial plants. Its spherical virions of ~50 nm in diameter consist of two dsRNA segments (dsRNA1, 8,931 bp and dsRNA2, 7180 bp). DsRNA1 is composed of open reading frame (ORF)1 and ORF2 that encode capsid proteins and RNA-dependent RNA polymerase, while dsRNA2 has two ORFs that may code for proteins with unknown functions. Importantly, RnMBV1 reduces the virulence of *R. necatrix*, thus making RnMBV1 a potential virocontrol agent. In the laboratory, we isolated mutant strains of RnMBV1 (RnMBV1-M), after transfection with wild-type RnMBV1, that retained two dsRNA segments, dsRNA1 and newly emerging dsRNA3, but not dsRNA2. Sequencing and Northern hybridization analyses of two variants of dsRNA3 (3a and 3b) revealed that they both originated from dsRNA1 by almost complete duplication of ORF2, tandemly arranged. The difference between dsRNA3a and 3b was the length of ORF2 retained. Purified virions of RnMBV1 and RnMBV1-M were similar in size and in infectivity of *R. necatrix* when tested by transfection into host protoplasts. However, transfectants with RnMBV-M showed restored colony growth, melanization, and virulence on apple tree roots compared to transfectants with wild-type RnMBV1. The different reactions of *R. necatrix* to infections with wild-type and mutant RnMBV1 strains could provide some clues to elucidate the mechanism of virulence in *R. necatrix*.

PS03-163

Characterization of *CoIRA1* of *Colletotrichum orbiculare*, required for infection-related morphogenesis and pathogenicity

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Through *Agrobacterium tumefaciens*-mediated transformation (AtMT) of *Colletotrichum orbiculare* strain 104-T (MAFF240422), an anthracnose disease fungus of cucumber, a mutant named AA4510, which showed abnormal infection-related morphogenesis and attenuated pathogenicity was previously isolated. Analysis of the mutation confirmed an insertion into a gene which putatively encodes 2255-amino acid protein with a predicted RAS GTPase-activating protein (RASGAP) domain. And we named this gene as *CoIRA1*. In human, the neurofibromatosis type 1 (*NF1*) gene encodes the GTPase-activating protein (GAP) neurofibromin, which negatively regulates Ras activity. *Saccharomyces cerevisiae* has two neurofibromin homologs, *Ira1* and *Ira2*. Targeted gene deletion mutants of *CoIRA1* indicated that *CoIRA1* is involved in proper appressorium development and penetration hyphae development. Appressoria produced by *coira1* disruption mutants showed irregular shape of appressoria on glass slides. And *coira1* disruption mutants develop bulb shape penetration hyphae into cellulose membrane unlike tubular form of the wild type. Since *CoIRA1* is involved in infection-related morphogenesis, we investigated whether *CoIRA1* is required for pathogenicity. The *coira1* mutants showed reduced pathogenicity on the cucumber leaves compared with the wild type. However, the *CoIRA1* complemented *coira1* mutants restored their ability to form proper appressoria, penetration hyphae and pathogenicity. In conclusion *CoIRA1* is involved in abnormal infection-related morphogenesis and reduction of pathogenicity in *Colletotrichum orbiculare*.

PS03-164

Functional characterization of genes encoding forkhead transcription factors in *Magnaporthe oryzae*

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Forkhead-box protein is a transcription factor (TF) playing critical roles in a broad spectrum of cellular processes. In *Saccharomyces cerevisiae*, 4 forkhead TFs, named FKH1, FKH2, HCM1, and FHL1, control cell cycle progression and rRNA processing. In filamentous fungi, however, little is known about functional roles of forkhead TFs. We identified 4 forkhead TFs from the genome of the rice blast fungus, *Magnaporthe oryzae*. Phylogenetic analysis revealed that among 4 putative forkhead TFs of *M. oryzae*, two were yeast-related (MoFKH1 and MoHCM1) and the others were filamentous fungi-specific (MoFOX1 and MoFOX2). Δ*Mofkh1* showed abnormal septation both in conidia and mycelia. The increased number of septa and nucleus in conidia implied the uncontrolled cell division. Furthermore, Δ*Mofkh1* exhibited low frequency of conidial germination and was more sensitive to several stress conditions, resulting in reduced virulence. Δ*Mohcm1* was sensitive to spindle poison, benomyl, analogous to yeast Δ*hcm1* indicating the possible role in chromosome segregation. On the other hand, Δ*Mofox1* was indistinguishable in several phenotypes including pathogenicity from those of wild-type. MoFOX2 seemed to be an essential gene since no Δ*Mofox2* was not generated from repeated attempts of targeted gene deletion. These results suggest

that yeast-related forkhead TFs in *M. oryzae* function in cell cycle regulation demonstrating their similarities to yeast homologs and MoFKH1 also plays an important role on fungal pathogenicity.

PS03-165

***Colletotrichum orbiculare CoBUB2*, the homolog of *Saccharomyces cerevisiae BUB2*, is involved in proper nuclear distribution, morphogenesis, and pathogenesis**

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Positioning of the mitotic spindle with respect to the polarity axis becomes important to complete proper nuclear division in *Saccharomyces cerevisiae*. A surveillance mechanism named spindle position checkpoint (SPOC), monitors the orientation of the mitotic spindle and prevents cells from exiting mitosis in response to spindle orientation defects. *Agrobacterium tumefaciens*-mediated transformation (AtMT) was used to generate morphogenesis deficient mutants in *Colletotrichum orbiculare*. From a mutant coQ-1 T-DNA inserted gene region was identified and sequenced, and the predicted coded amino acid sequence showed high conservation to that of *BUB2* in *S. cerevisiae*, a component of SPOC. We named this gene *CoBUB2*, and gene knock-out mutants were generated by AtMT and observed infection related morphogenesis. The conidial shape of *cobub2* mutants was more spherical compared with wild-type. The appressoria forming conidia of *cobub2* mutants developed germinated hyphae from conidia with high frequency. The penetration hyphae of *cobub2* mutants showed abnormal morphology and crept on the surface of cellulose membranes used as a model substrate. The *cobub2* mutants showed attenuate pathogenesis to cucumber leaves, indicating that *CoBUB2* is required for full pathogenesis. Since *BUB2* is a component of SPOC, we observed the nuclear behavior of *cobub2* mutants by DAPI. While wild-type contained single nuclear in single cell, the *cobub2* mutants frequently contained two nuclei in single cell in a process of appressorium development. In conclusion, there could be a mechanisms controlled by SPOC that regulates coordinates processing of nuclear division and appressorium development in *C. orbiculare*.

PS03-166

Functional analysis of the bZIP transcription factor family in *Magnaporthe oryzae*

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Lifestyle of fungi depends largely on their adaptability in environments. It is therefore crucial to elucidate the transcriptional programs operating under different environmental conditions such as physical and chemical stresses, and host-dependent constraints. Regulatory roles of the basic leucine zipper (bZIP) transcription factors (TFs) in fungi have been identified in diverse cellular processes such as nitrogen metabolite repression, iron supply, sulfur metabolism, and other various stress responses. In this study, genes encoding bZIP (*MoZIPs*) TF family in the rice blast fungus, *Magnaporthe oryzae* has been systemically characterized. bZIP TF sequences from 36 fungal species were identified and analyzed for their phylogenetic relationship. In total, 12 clades encompassing MoZIPs and conserved orthologs were identified only in phylum Ascomycota. Quantitative RT-PCR analysis for all *MoZIPs* on 32 different conditions showed dynamic expression profiles, suggesting their involvement in various stress responses and during pathogenesis. To link phylogenetic and expression data to phenotypes, gene deletion mutants were generated for 9 *MoZIPs* having orthologs, and 4 *Magnaporthe*-specific ones. Among total 13 deletion mutants, 3 show functional conservation with their characterized orthologs and detectable phenotype changes on growth in several stress conditions, developments and/or pathogenicity were observed from other 6 mutants. Deletion of other 4 genes does not make any distinguishable changes compared to the wild-type. Taken together, MoZIPs play critical roles in adapting environmental changes, fungal development and pathogenicity, especially highly conserved members reflecting their functional importance.

PS03-167

Identity and distribution of ORFs from non-tox common regions of ACT- and AF-toxin Tox chromosomes among various isolates of *Alternaria alternata*

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The tangerine pathotype of *A. alternata* produces host-selective ACT-toxin and causes Alternaria brown spot disease of tangerines and mandarins. AK- and AF-toxins are also HSTs produced by the Japanese pear and strawberry pathotypes of *A. alternata*. A structural part of ACT-, AK- and AF-toxins shares a common moiety of 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid. Homologues of 6 genes responsible for the biosynthesis of decatrienoic acid moiety in the tangerine pathotype were also found in the Japanese pear and strawberry pathotypes. We identified total of 10 ACT-toxin biosynthesis genes and characterized their functions in ACT-toxin production. These genes are located in ACT-toxin Tox (*ACTT*) cluster in the small chromosome with the size of 1.9 Mb. Mass sequencing of the 1.9 Mb chromosome using Roche 454 GS-FLX System identified 49 contigs including the largest continuous sequence of 618,946 bp. We analyzed similarity of these sequences against that of the 1.05 Mb chromosome containing AF-toxin Tox (*AFT*) cluster of the strawberry pathotype. Addition to the similarity found in the Tox clusters of both pathotypes, about 200 kb region containing 43 ORFs apart from the Tox clusters also showed similarity and more than 80% sequence identity was found in 9 ORFs. Distribution and identity analysis of these 9 ORFs among various isolates of tangerine pathotype as well as other pathotypes of *A. alternata* identified that some of the ORFs might be used as monitoring sequences for identification of Tox chromosome among HST-producing *A. alternata*.

PS03-168

Isolation and characterization of soil microorganisms with anti-*Ganoderma* properties

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Ganoderma boninense is a pathogenic fungus of economically important crops, such as oil palm, coconut, rubber and betelnut. In South-East Asia, it is the main causative agent of basal stem rot (BSR) disease of oil palm. The devastating effect of BSR disease has led to the oil palm industries demand for an immediate cure. Among the *Ganoderma* spesies, *G. boninense* is known to be the most aggressive. Recent statistics show that the BSR disease incidents are increasing at the younger and productive stage of oil palm, especially in replanted area, and coconut or oil palm areas underplanted with young oil palm. In this study, the fruiting bodies of *G. boninense* were collected from infected oil palm

trees, at Kulai, Johor, Malaysia. To isolate bacteria and fungi that can be used as potential biocontrol agent for BSR disease, soil microorganisms were isolated and screened for anti-Ganoderma activity. Growth inhibition test against *G. boninense* was conducted. Various microorganisms were direct inoculated on Mueller Hinton agar pre-swabbed with liquid culture of*G. boninense* growing at the vegetative stage. Three bacterial isolates were found to antagonize the growth of *G. boninense* that formed clear inhibition zones. Using API biochemical kits, these bacterial isolates were identified as *Bacillus subtilis*, *Actinomyces israelii*, and *Staphylococcus saccharolyticus*. Further microscopic examination and biochemical activity analysis of the isolates were conducted.

PS03-169

Genetic studies of the sugarcane smut fungus *Sporisorium scitamineum*

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Teliospores of *Sporisorium scitamineum*, the fungus that causes sugarcane smut, were obtained from infected plants of varieties of sugarcane grown in different areas in Brazil. Whips from 38 plants were collected and teliospore-derived mycelial colonies were obtained. Single haploid yeast-like cells were randomly isolated from each teliospore-derived colony. Plate mating experiments for determination of plus or minus mating-type were performed. A combination of light and scanning electron microscopy was used to examine teliospores and teliospore germination, confirming the presence of the characteristic scattered echinulations. Chromosome characterization was achieved based on pulse field gel electrophoresis indicating the presence of 20 chromosomes ranging from 144 to 2200 Kbp, resulting in an estimate of the genome size of approximately 20 Mbp. AFLP and telomere-based RFLP revealed polymorphisms among haploid cells derived both from the same teliospore as well as from distinct teliospores.

PS03-170

De novo partial genome assembly of the biotrophic eucalyptus rust pathogen *Puccinia psidii*

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The genus *Puccinia* is considered to contain the most destructive genera of biotrophic fungi. *P. psidii* which causes the *Eucalyptus rust* (up to 60% losses in young plants). Despite of its economic importance, little is known about the biology of the fungus and its molecular interaction with the host. We sequenced *P. psidii* genomic DNA obtaining more than 90,000 random genomic contigs (average size 1500 bp). The largest 19,500 contigs were annotated using the Blast2GO tools and almost 50% of the sequences are associated with DNA metabolism, which includes retro-transposons, which are highly represented within the fungal genome. The second most abundant GO term was associated with catabolism, includes a large proportion of glycoside hydrolases from different families, some of which are related to pathogenic processes (for instance GH3, GH5, GH16, GH18, GH61). We also found siderophores and some interesting secondary metabolites. Other sequences were associated with reproduction, cell cycle and differentiation, signal transduction, responses to stress and other classes that will help to improve our knowledge of fungal biology. Currently, just three *Puccinia* genomes are in the process of being sequenced and are publicly available: *P. graminis*, *P. triticina* and *P. striiformis*, thus our data is the first set of useful genomic sequence information from *P. psidii*. Although the sequence assembly is only of a draft quality, it is a valuable resource for generating working hypothesis about the fungal biology and the interaction between eucalyptus-*P. psidii* aiming at the development of strategies to control *Eucalyptus* rust in this crop.

PS03-171

Roles of rice transcription factor OsWRKY76 in response to the rice blast fungus

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OsWRKY76, a rice WRKY transcription factor responds to infection by the blast fungus *Magnaporthe oryzae* at a transcriptional level. We have characterized the role of OsWRKY76 in disease responses. OsWRKY76 belongs to the WRKY group IIa. GFP-fused OsWRKY76 was localized to the nucleus in onion epidermal cells. OsWRKY76 had transcriptional repressor activity in rice cultured cells. Gel mobility shift assay demonstrated that purified recombinant OsWRKY76 protein bound to W-box elements in vitro. Real-time PCR analysis showed that expression of *OsWRKY76* was induced by wounding, low-temperature, and application of BTH and ABA. Transgenic rice plants overexpressing *OsWRKY76* increased susceptibility to the compatible rice blast fungal races. The transgenic plants also showed reduced tolerance to an incompatible race. In contrast, the transgenic plants showed slightly improved tolerance to low-temperature stress. Therefore, *OsWRKY76* is suggested to be involved in biotic and abiotic stress responses. To identify target genes of OsWRKY76, we compared gene expression profiles between wild-type and *OsWRKY76*-overexpressing transgenic plants in response to inoculation of blast fungus by microarray analysis. The transgenic plants showed impaired transcriptional induction of several pathogenesis-related (PR) genes such as *PR1*s, *PR2*s, *PR5*s, *PR9*s, and *PR10*s. In addition, overexpression of *OsWRKY76* caused reduced expression of genes encoding enzymes responsible for the synthesis of diterpenoid phytoalexins. These genes contain W-box-like elements in their promoter region. These results suggested that OsWRKY76 suppresses disease resistance via down-regulation of these defense-associated genes.

PS03-172

Novel MAP kinase signaling cascade in *Arabidopsis* resistance to mycotoxigenic fungi

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Mitogen-activated protein kinase cascades play important roles of immune response in animals and plants. Many MAPKs and MAPKKs were known to be involved in defense system of plant, whereas functions of most MAPKKKs were largely unknown. A phytopathogenic fungus, *Fusarium sporotrichioides* produced a lethal mycotoxin, T-2 toxin in host plants. T-2 toxin also acts as a phytotoxin and induced cell deaths through prolonged activation of some MAPKs in *Arabidopsis thaliana*. However, it was unknown that a MAP kinase cascade regulating a phytotoxin response and disease resistance to *F. sporotrichioides*. Here we show that a novel MAPKKK, MKD1 was identified as one of subunits of AtNFXL1 complex and positively regulated disease resistance to *F. sporotrichioides*. MKD1 directly interacted with MKK1 and MKK5 *in vivo* and phosphorylated these MKKs *in vivo*. In

addition, the activation of MPK3 and MPK6 by T-2 toxin was apparently decreased in *mkd1* mutant compared with wild type. Correspondingly, *RNAi:MKK5* transgenic plants and *mkk1* mutant showed enhanced susceptibility phenotype to *F. sporotrichioides*. Finally, quantitative proteomics of phosphorylated proteins revealed that phosphorylation of defense-related proteins such as SUMOs, a disease resistance protein, and a mycotoxin-detoxifying enzyme, were controlled by the MKD1-dependent signaling cascade. Thus, we revealed that the MKD1 phospho-signaling pathway play important roles in the disease resistance against mycotoxigenic *F. sporotrichioides*.

PS03-173

Characterization of isolates of *Alternaria* spp. recovered from apple in Italy

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Since 1999, a disease of apple caused by an *Alternaria* sp. was reported in North Italy (Trentino Alto Adige region). By 2002 the disease broadened to other regions and nderstanding the genetic diversity and the distribution of *Alternaria* spp. became a fundamental step in controlling the disease. A comparison with *Alternaria alternata mali* pathotype strains has been carried out to investigate the relationship of the Italian pathogen to the causal agent of the Apple Blotch disease. A morphological characterization was conducted, describing the 3-dimensional sporulation pattern and the colony morphology of each strain include un this study. To assess the genetic diversity within the Italian *Alternaria* population, sequence characterization of one protein coding gene and three non-coding regions and genetic fingerprinting based on AFLP and ISSR markers were performed. The pathogenicity was tested with three bioassays and showed differential capability of the isolates for causing disease, which did not correlate with the morphological groupings or to groupings defined by molecular approaches. Ten pathogenic isolates were positive for the AMT toxin gene, a specific pathogenicity factor, based upon PCR amplification using specific primers for the AMT gene. This suggests that the production of the host specific AMT toxin may be involved in pathogenesis by some of the Italian isolates of *A. alternata* from apple. This research suggests that a number of different *Alternaria* genotypes and morphotypes may be responsible for the Italian apple spot disease and that a single taxon cannot be defined as the sole causal agent.

PS03-174

Genome-wide analysis of Pox genes in fungi

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Peroxidase (Pox) genes are known to play key roles in cell signaling and transduction, production/detoxification of reactive oxygen species (ROS) and also in the pathogenicity of fungi. Using bioinformatic approaches we conducted a comparison of 20 different Pox gene families genome-wide on 30 different species, including plant/animal fungal pathogens, model and forestry fungi. Our results show that especially the copy number of the ancestral NADPH oxidase gene family is in most pathogens 10 ± 3 while in most non-pathogens 6 ± 3. This could indicate that most pathogens require an efficient detoxification system for host generated ROS. To our knowledge this is the first attempt of a genome-wide analysis of Pox genes between 30 different fungal species. In the future this data will be used for further investigation of specific Pox genes in wet-lab experiments.

PS03-175

TranscriptomeS of *Botrytis cinerea*

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Botrytis cinerea is a polyphageous and necrotrophic ascomycete responsible for grey mould on grapevine and more than 200 other plant hosts. The complete sequencing of its genome allowed the annotation of approx. 14000 genes and the design of Nimblegen arrays dedicated to transcriptomics studies (Amselem et al., PLoS Genetics, 2011). We used this approach to compare the transcriptomes of *B. cinerea* in distinct physiological conditions including *in planta* development (different hosts) and *in vitro* growth (different media). Clustering all the experiments together highlighted the similarities between some physiological conditions *e.g.* growth on a solid grape juice medium and late infection stages on grape berries. Comparing the different transcriptomes also allowed the identification of genes that are specifically induced during the infection process such as the clusters responsible for the biosynthesis of the phytotoxins botrydial and botcinic acid (Dalmais et al., MPP, 2011). Additional unknown sesquiterpens and polyketides were identified as putative virulence factors. Other functional categories of genes related to oxidative stress, carbohydrate-active enzymes and transcriptional regulation were highlighted. Functional characterizations of several putative fungal virulence factors are in progress. NB: A part of the presented data were generated through the *SafeGrape* project founded by the ANR (French National Agency for Research) and the CNIV (Comite National des Interprofessions des Vins a A. O.). Nimblegen arrays hybridizations were performed by PartnerChip (Evry, France).

PS04-176

The trans-Golgi network/early endosome is a critical endomembrane organelle for the execution of plant stress responses

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In plants, the *trans*-Golgi network (TGN) includes early endosomes (EE) and functions as a sophisticated sorting system for directing vesicles and associated proteins coming from the Golgi or plasma membrane to vacuoles or back to the plasma membrane. Our study on several molecular components of the TGN/EE indicates that this organelle plays an essential role in regulating response to biotic and abiotic stress. We determined that KEG (*Keep On Going*) is an integral component of TGN/EEs and plays an important role in regulating secretory trafficking and vacuolar targeting of membrane proteins. Mutations in KEG suppressed *min7* (*HopM1 interactor 7*)- and *edr1* (*Enhanced Disease Resistance 1*)-mediated programmed cell death under various stress conditions. Consistent with this genetic interaction, we found that KEG co-localizes with MIN7, which is an ADP ribosylation factor-guanine nucleotide exchange factor (ARF-GEF) that acts at multiple steps of the secretory and vacuolar trafficking pathways. In addition, we determined that KEG recruits EDR1 to the TGN/EE through its C-terminus HERC2-like repeats. Overexpression of KEG enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato*. However, KEG was degraded specifically in cells infected by the virulent powdery mildew pathogen *Golovinomyces cichoracearum*, suggesting that this pathogen may target KEG for degradation to suppress secretion of defense proteins. Collectively, these data provide evidence for an important link between endomembrane trafficking regulators and plant stress responses.

PS04-177

The use of the soils fungus *Penicillium canescens* in the increase the harvest to Soybean plants

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At symbiosis of the plant with soil fungus receive additional feeding in the manner of elements. A soil fungi *Penicillium* sp. capable of solubilizing of Phosphorus components from difficult-available form to the easy form the phosphates. The fungus gifes penetrate inside fabric root system of the high plants and actuate the influx mineral material in root. The plant in turn delivers the fungus ready organic nutrients. The plants of soya enter in symbiosis with nodule bacteria *Bradyrhizobium japonicum* and for in their own root nodule and provide them with nitric feeding. These both processes - the assimilation of the nitrogen and phosphorus are checked controlled by host plants. The experiments were conducted in the Greenhouse condition. Seeds were surface sterilized and inoculated with *Bradyrhizobium japonicum* and growing special pots which contained (sterile) sand with NPK components contained fungus inoculume. Plants were cultivated 30-45 day. The conducted the observation on the plants by comparing and checked the experience variants with the control variants was studied. The dry weights shoot and root of plants in the experiments variants. Were above than with control variants. The two types of microorganisms do not appear to compete for nutrients in the rhizosphere of legumes and the *Penicillium* ssp provides a source of available phosphorus for use by the plant without adversely affecting the nitrogen fixation ability of the *Rhizobium* spp. and indeed such ability is enhanced.

PS04-178

A barley RAC/ROP interacting ROP binding kinase (HvRBK1) influences microtubule stability and is involved in pathogen response to the barley powdery mildew fungus

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Small monomeric G proteins of the plant specific Rho family, called Rho of plants (RAC/ROPs) are involved in a variety of signaling processes like plant development, cytoskeleton remodeling and pathogen defense. Amongst others, active RAC/ROPs interact with receptor-like cytoplasmic kinases (RLCKs or ROP binding kinases, RBKs) *in planta* and regulate their activity *in vitro*. Although downstream kinases are prominent modulators in pathogen signal transduction mechanisms, the function of RBKs in plant immunity is not yet understood. Here, we report the identification of a barley RAC/ROP interacting ROP binding kinase (HvRBK1) in yeast and *in planta*. HvRBK1 shows basal kinase activity which is increased in the presence of HvRACB and HvRAC1. We demonstrated that GFP-tagged HvRBK1 is located in the cytoplasm of barley epidermal cells and gets recruited to the plasma membrane upon co-expression of activated HvRACB or HvRAC1 respectively. Furthermore, transient induced gene silencing of HvRBK1 influences stability of microtubules (MT) in barley epidermal cells and enhances penetration success of the parasitic fungus *Blumeria graminis f.sp. hordei*. In summary, our results suggest a function of barley RBK1 in basal resistance to powdery mildew.

PS04-179

Secretion of effector proteins in rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is a devastating plant pathogenic fungus which causes blast disease in a broad range of cereals and grasses. *M. oryzae* secretes a repertoire of effector molecules which alter host plant metabolism and suppress defence responses. It is known that exocytosis in polarized filamentous fungi happens through the hyphal tip and the secretion of most proteins requires the conventional Endoplasmic Reticulum (ER)-Golgi pathway. It had been thought that the secretion of effectors will follow the same mechanism. Recent studies in *M. oryzae* have, however, shown that effectors accumulate in a novel structure called the Biotrophic Interfacial Complex (BIC). This is a membrane rich structure at the interface between the host plant cell and fungal invasive hyphae. The structure and function of BICs is unknown and the underlying secretory apparatus is also unidentified in *M. oryzae*. We are currently characterizing components of exocyst and SNARE complex that may be necessary for secretion of effector molecules. Furthermore, our results suggest that effector secretion may not necessarily require the conventional ER-Golgi pathway. We aim to define the involvement of the exocyst and function of conventional secretory mechanism in effector delivery and then determine whether novel secretory processes might have evolved in the fungus to allow delivery of effectors directly into the host plant cells.

PS04-180

Surface-mediated response to elicitins is providing a novel layer of resistance to *Phytophthora infestans* in potato

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Surface-mediated response is based on pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs). In contrast to resistance genes that are commonly used in resistance breeding and generally quickly defeated, PRR are reported to confer a broader type of recognition. Recently, it was shown that PAMP-triggered immunity can confer a broad-spectrum disease resistance in crop plants. This suggests that PRR have great promise for engineering effective and durable disease resistance. In this project, we study the *ELR1* gene, which encodes the first potato PRR that recognizes elicitins of the potato late blight pathogen *Phytophthora infestans*. Since elicitins are widely conserved and recognized as oomycete PAMPs, a defense response targeted to elicitins is expected to be generally broad spectrum. This hypothesis is confirmed by our results. We tested 16 elicitins from 8 different oomycete species including *P. infestans*, and found that most elicitins were recognized by ELR1. Besides, we are testing whether expression of *ELR1* in potato can enhance the resistance to *P. infestans* isolates. We will report on these data during the conference and discuss whether *ELR1* can potentially confer an enhanced broad-spectrum resistance to late blight.

PS04-181

Identification of a hidden resistance gene in tetraploid wheat using laboratory strains of *Magnaporthe oryzae* produced by backcrosses

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PWT3 is a gene involved in the avirulence reaction of *Avena* isolate Br58 of *Magnaporthe oryzae* on wheat. Molecular mapping using BC_1F_1 population derived from the backcross 73Q2 and the wheat isolate Br48 revealed that *PWT3* locus is located on chromosome 6 and completely linked to an SSR marker MoSSR6-1. White and black colonies segregated in a 1:1 ratio using BC_3F_1 population, suggesting that colony color is controlled by a single gene. The progeny are considered color mutants because both parental cultures are black. Colony color is perfectly linked with virulence of the BC_3F_1 population on wheat cultivar Norin 4. A cross between a moderately resistant tetraploid cultivar Tat4 and susceptible tetraploid cultivar Tat14 to the white BC_3F_1 cultures produced F_2 seedlings which segregated 3:1 ratio, suggesting that resistance is also controlled by a single gene. This gene was designated as *RmgTat4* and is considered a hidden resistance gene because it was not detected with Br58, F_1, BC_1F_1 and BC_2F_1 isolates. Molecular mapping using F_3 lines derived from the cross Tat4 and Tat14 revealed that *RmgTat4* is located on chromosome 7B. Cytological analysis showed that Tat4 produced hypersensitive reaction of mesophyll cells upon inoculation with a BC_3F_1 isolate.

PS04-182

Expression of the late blight resistance gene *Rpi-phu1* after the pathogen challenge

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Since the first half of the twentieth century, when eleven resistance genes (*R1-11*) against *Phytophthora infestans* from *Solanum demissum* were discovered, a lot of new R-genes have been identified in wild species of *Solanum* genus. Among them, there was also the *Rpi-phu1* gene, identified in *S. phureja* and mapped to potato chromosome IX. The *Rpi-phu1* gene was transferred into cultivated potato gene pool using a series of interspecific crosses, first at the diploid, and then tetraploid level. Plants with *Rpi-phu1* are used as differential in characterizing the Polish *P. infestans* population. Later, its sequence was shown to be identical with *Rpi-vnt1.1* sequence. The aim of the ongoing experiments was to investigate expression pattern of the *Rpi-phu1* gene in the non-infected and infected plants of different diploid and tetraploid genotypes. Influence of plant age on *Rpi-phu1* gene expression was also investigated. Specific PCR marker located within the sequence of the *Rpi-phu1* gene was designed and optimized. α-tubulin was chosen as a reference gene. Samples were taken from plants before inoculation and 1, 3, 5 days after. Relative expression of *Rpi-phu1* was measured in five biological and three technical replications. The genetic background and plants ploidy had significant effect on the *Rpi-phu1* expression level. Young, 3-week-old plants differed significantly in their *Rpi-phu1* expression pattern from 6, 9 and 12-week-old plants.The research is founded by Polish NCBiR grant LIDER/06/82/L-1/09/NCBiR/2010 and PW 3-1-06-0-01.

PS04-183

Transcriptome analysis of hexaploid wheat in the early stages of floral colonisation by the ergot fungus *Claviceps purpurea*

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The ergot fungus, *Claviceps purpurea* , is a broad host range pathogen which readily infects grass flowers around anthesis by adhering to stigma hairs and following a pathway to the base of the ovule. This closely mimics the penetration of the pollen tube but instead of forming a grain, a toxic sclerotia is produced. We have performed Illumina transcriptome sequencing of dissected wheat carpel tissue from 10 minutes to 7 days after inoculation (DAI), and will present the most comprehensive wheat floral transcriptomics results to date. We shall be asking what is the extent of PAMP-triggered immunity in this susceptible interaction? At 3 DAI genes like the CEBiP are up-regulated, but what others will match the expression pattern? We have found that genes regulating plant hormones have been amongst the most differentially regulated at 3 DAI and will therefore piece together the wider implications if this. We have included several libraries tracking the route of pollen tubes, to ascertain any similarities of the plant response to pollen and hyphae. Using the *Claviceps* genome sequences as reference we have also isolated the fungal transcripts and will present expression changes that occur at key points in the fungal lifecycle from the germination of conidia to the rapid branching and colonisation that occurs at 3-4 DAI, and subsequent production of conidia in honeydew, and finally the time at which the alkaloid biosynthesis genes are turned on.

PS04-184

Identification of flagellar mastigoneme proteins from *Phytophthora*

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Motile, flagellate zoospores of *Phytophthora* and *Pythium* species play a key role in pathogen dissemination and the initiation of infection of host plants. The diseases these pathogens cause are highly destructive and result in extensive losses in agriculture and natural ecosystems worldwide. Tripartite tubular hairs called mastigonemes on the anterior flagellum of *Phytophthora* and *Pythium* and other protists in the Stramenopile taxon are responsible for reversing the thrust of flagellar beat and for cell motility. Immunoprecipitation experiments using antibodies directed towards mastigonemes on the flagella of zoospores of *Phytophthora nicotianae* have facilitated the identification of three similar proteins. A gene for one of these proteins has been cloned and encodes a mastigoneme shaft protein. Expression of the gene, designated *PnMas2*, is up-regulated during asexual sporulation, a period during which many zoospore components are synthesized. Analysis of the sequence of the PnMas2 protein has revealed that, like other Stramenopile mastigoneme proteins, PnMas2 has an N-terminal secretion signal and contains four cysteine-rich epidermal growth factor (EGF)-like domains. Evidence from non-denaturing gels indicates that PnMas2 forms large oligomeric complexes, most likely through disulphide bridging. Bioinformatic analysis has revealed that Phytophthora species typically have three or four putative mastigoneme proteins containing four EGF-like domains. These proteins are similar in sequence to mastigoneme proteins in other Stramenopile protists including the algae *Ochromonas danica*, *Aureococcus anophagefferens* and *Scytosiphon lomentaria* and the diatoms *Thalassiosira pseudonana* and *T. weissflogii*.

PS04-185

***In vivo* expression system for effector validation in hexaploid wheat (*Triticum aestivum* L.) using protoplast electroporation**

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Wheat (*Triticum aestivum* L.) is severely affected by cereal rusts. Rust effectors secreted during the infection induce the uptake of

nutrients and neutralize the host defense responses. Major *R* genes can recognize specific pathogen effectors (*Avr*) in a gene-for-gene manner and trigger a hypersensitive response. Although *R* genes are used in breeding programs to develop resistant varieties, the fungus tends to overcome these new resistance sources shortly after deployment. Identification and characterization of *Avr* genes from cereal rusts is critical to fully understand pathogenicity and generate new strategies for disease management. Despite their importance no *Avr* gene from cereal rusts has been cloned due in part to a complex biotrophic nature of the pathogen. Although progress has been achieved in computational prediction of potential candidates, functional validation of effectors is still critical to identify their role during infection. Here we report protoplast isolation from wheat etiolated seedling and the transient expression of luciferase after electroporation. We evaluated different wheat varieties and different conditions (temperature, time after electroporation, culture media) to improve the efficiency of the transformation and provide a reliable method for a rapid characterization of a large number of effectors. Results of these approaches and the validation of the method with a positive control will be discussed

PS04-186

Development of gel and LC-MS/MS based method for proteomics analysis of pathogen induced response in *Malus* sp.

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Research on interaction of *Malus* sp. and *V. inaequalis*, the causal agent of the apple scab disease, presents a comprehensive knowledge on biology and genetics of resistance to fungal pathogens in apple. However, understanding of mechanistic basis of the resistance to apple scab and other fungal pathogens remains scarce. *Malus* sp. has been designated as one of three model species of the *Rosaceae* family and extensive genome information became available for genomics analysis of plant responce, recently. Proteomics has been proven an effective approach in studies on regulation of biological processes at post-translational level. However, differential analysis of expression of proteins involved in plant defense response pathways requires highly sensitive and robust technique. Therefore, the aim of this study was to develop a proteomics method for analysis of pathogen induced response in *Malus* sp. Apple tree leaf and *in vitro* grown apple microshoot tissue samples were used in the study. We pre-fractionated the tissue into soluble cytosolic, microsomal, nuclear and organellar protein fractions to reduce complexity of the sample and to enhance sensitivity. Application of saturating labeling with rhodamine-based fluorescent dyes provided sensitive detection of proteins using 2DE technique. Membrane proteins of microsomal fraction were separated by 1D-PAGE and tryptic peptides were analyzed using LC-MS/MS following in-gel digestion. Sensitivity and specificity of the method for detection of proteins involved in stress response pathways is being assessed using samples prepared from apple leaves treated with salicylic acid or *V. inaequalis* culture filtrate.

PS04-187

Alternative splicing of a multi-drug transporter from *Pseudoperonospora cubensis* generates an RXLR effector protein that elicits a rapid cell death

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Pseudoperonospora cubensis, an oomycete pathogen, is the causal agent of cucurbit downy mildew. Similar to other oomycete plant pathogens, *Ps. cubensis* has a suite of RXLR and RXLR-like effector proteins which likely function as virulence or avirulence determinants during the course of host infection. We identified 271 candidate effector proteins within the *Ps. cubensis* genome with variable RXLR motifs. We also present the functional characterization of one *Ps. cubensis* effector protein, RXLR protein 1 (PscRXLR1), and its *Phytophthora infestans* ortholog, PITG_17484, a member of the Drug/Metabolite Transporter (DMT) superfamily. To assess if such effector-non-effector pairs are common among oomycete plant pathogens, we examined the relationship(s) among putative ortholog pairs in *Ps. cubensis* and *P. infestans*. Of 271 predicted *Ps. cubensis* effector proteins, 109 (41%) had a putative ortholog in *P. infestans* and evolutionary rate analysis of these orthologs shows that they are evolving significantly faster than most other genes. PscRXLR1 expression was up-regulated during the early stages of infection of plants, and, moreover, that heterologous expression of PscRXLR1 elicits a rapid necrosis. More interestingly, we also demonstrate that PscRXLR1 arises as a product of alternative splicing,making this the first example of an alternative splicing event in plant pathogenic oomycetes transforming a non-effector gene to a functional effector protein. Taken together, these data suggest a role for PscRXLR1 in pathogenicity, and, in total, our data provide a basis for comparative analysis of candidate effector proteins and their non-effector orthologs as a meansof understanding function and evolutionary history of pathogen effectors.

PS04-188

Characterization of regulated protein secretion in *Phytophthora* zoospores

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In many species of *Phytophthora* and other oomycetes, motile biflagellate zoospores initiate plant infection. Within the zoospore cytoplasm, organelles, including three types of cortical vesicles, are distributed with a distinct polarity. Rapid exocytosis or migration of these vesicles during zoospore encystment indicates that the cortical vesicles may play important roles during early infection. Within the first 2 minutes of zoospore encystment, the contents of ventral and dorsal cortical vesicles are secreted, delivering adhesives and a putative protective coating onto the surface of the cysts. By contrast, the third category of cortical vesicles, the large peripheral vesicles, move away from the plasma membrane, become randomly distributed within the cyst cytoplasm and ultimately degraded. Unexpectedly, we found that PnCcp, a 12 kDa protein component of the large peripheral vesicles is somehow selectively secreted during encystment. Double immunolabelling studies have shown that in sporangia and zoospores, PnCcp colocalises with PnLpv, a high molecular weight glycoprotein also resident in the large peripheral vesicles. However, in hyphae, the large peripheral vesicles sometimes contain only PnLpv and quantitative analysis suggests that during vesicle development, PnCcp is added to large peripheral vesicles after PnLpv. Quantitative, real-time RT-PCR shows that expression of *PnLpv* precedes that of *PnCcp* and that *PnCcp* but not *PnLpv* is expressed in zoospores. PnCcp and PnLpv were also found to be differentially compartmentalised within the vesicles. The differential synthesis and secretion of large peripheral vesicle proteins in *Phytophthora* zoospores provides a novel system in which to study selective protein secretion in eukaryotes.

PS04-189

Characterisation of gene families encoding cell wall degrading enzymes in *Phytophthora*

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Apoplastic effectors produced by plant pathogenic bacteria, fungi and Oomycetes include a broad spectrum of enzymes that degrade the plant cell wall during plant infection and colonization. Many pathogens have multiple genes encoding particular classes of cell wall degrading enzyme (CWDE). Species of highly destructive plant pathogens in the genus *Phytophthora* contain especially large CWDE multigene families. Contrary to early suggestions, the large size of CWDE gene families is not associated with a broad host range as *Phytophthora* species with both broad and narrow host ranges have similarly large numbers genes encoding different classes of CWDEs. The prevailing hypothesis is that large multigene families reflect the need for a range of substrate specificities within an overall enzyme class. The genomes of six *Phytophthora* species have now been sequenced. We have conducted an extensive bioinformatic analysis of the gene families encoding endopolygalacturonases, pectin methyl esterases and cellulases in these organisms. The analysis has contributed to refinement of genome annotation and identification of putative paralogous and orthologous genes. Documentation of the composition of the CWDE multigene families forms the basis for a detailed analysis of their expression during plant infection. Levels of expression of members of these gene families are being analysed using quantitative real-time PCR (qPCR) and RNAseq.

PS04-190

Genetic analysis of the incompatibility between *Lolium* isolates of *Magnaporthe oryzae* and wheat

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Lolium isolates of *Magnaporthe oryzae*, causal agent of gray leaf spot of perennial ryegrass (*Lolium perenne*), are virulent on perennial ryegrass but avirulent on wheat cultivars. To reveal genetic mechanisms of this specific parasitism, *Lolium* isolate TP2 was crossed with *Triticum* isolate Br48. Segregation analysis of their F_1 progenies revealed that the avirulence of TP2 on wheat cultivar Norin4 and Chinese Spring was conditioned by two unlinked aviruelence genes. One (tentatively designated as *A1*) was highly effective while the other (*A2*) was less effective. The resistance of the wheat cultivars to TP2 was also conditioned by two genes, one corresponding to *A1* and the other corresponding to *A2*. They were tentatively designated as *R1* and *R2*, respectively. The incompatibility between the *Lolium* isolate and wheat cultivars was conditioned by gene-for-gene interactions. *R1* was located on chromosome 7A by using chromosome substitution lines and designated *Rmg6*. Interestingly, *Rwt3*, a resistance gene in common wheat against *Avena* isolates of *M. oryzae* was also located on chromosome 7A. Furthermore, molecular mapping of the avirulence genes revealed that *A1* was located on a genomic region on chromosome 6 that contained *PWT3* corresponding to *Rwt3*. These results suggest that the *PWT3* may be commonly involved in the avirulence of two distinct subgroups of *M. oryzae* (*Lolium* and *Avena* isolates) on wheat.

PS04-191

Appressorium-localized NADPH oxidase B is essential for aggressiveness and pathogenicity in host specific toxin producing fungus *Alternaria alternata* Japanese pear pathotype

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Alternaria alternata Japanese pear pathotype is the causal fungus of black spot disease in Japanese pear. The spores germinate on pear leaves, extend their hyphae horizontally, form appressoria, and descend into host cells. This dramatic shift of cellular polarity at the apical hyphae is crucial to understand the pathogenicity. We have seen that Reactive Oxygen Species (ROS) were generated at plant-microbe interaction sites using transmission electron microscopy (TEM). Additionally, we found suppression of the ROS production using inhibitors (e.g., ascorbic acid and diphenyleneiodonium chloride) stopped pathogenicity. We also cloned two NADPH oxidase genes (NoxA and NoxB), presumable ROS generators, and found that only the *noxB* disruption mutant lost penetration ability, increased hyphal branching ratios, and lost pathogenicity regardless of AK-toxin production. Expression patterns of NoxA and NoxB did not vary greatly in any aspect of the life cycle as measured by quantitative RT-PCR. We also constructed NoxA/NoxB-mCherry-fusion protein and revealed that NoxB were localized at appressoria during infection process. Quantitative analysis of ROS production at plant surface-appressorium interaction sites by TEM showed that ROS were produced mainly on the pear leaves but little on the cellulose membranes although the fungus retained penetration ability, suggesting the necessity for some plant signal. These results indicate that appressorium-localized NoxB has an undiscovered role in the penetration other than ROS production.

PS04-192

The role of Nox complex components during pathogenicity of *C. purpurea*

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The ergot fungus *Claviceps purpurea* is a biotrophic phytopathogenic fungus with a broad host range including economically important crops like wheat, rye, and barley. Its infection behavior is highly organ specific as only ovaries of flowering grasses are infected. During the early infection stages *C. purpurea* shows polar growth mimicking the pollen tube. This behavior changes dramatically in the later stages where the whole ovarian tissue is colonized by frequently branched hyphae, finally producing the sclerotium. We are interested in the highly regulated differentiation process of *C. purpurea in planta* as well as in the signaling mechanisms to maintain this biotrophic interaction. One major aspect within this context is the NADPH-oxidase (Nox) complex.*C. purpurea* encodes two homologues of the mammalian gp91phox, *cpnox1* and *cpnox2*. The deletion of *cpnox1* leads to reduced infection rates with retarded honeydew production and immature sclerotia. In contrast, the deletion of *cpnox2* does not affect early colonization stages. However, Δcpnox2 shows enhanced and prolonged production of honeydew compared to the wildtype and sclerotia are even less developed than in Δcpnox1. We are investigating further putative complex components: the regulatory subunit CpNoxR as well as CpPls1, a tetraspanin often connected with CpNox2. In pathogenicity assays on rye ΔcpnoxR shows strong production of honeydew whereas Δcppls1 has a low infection rate. Both mutants produce small, not fully mature sclerotia. Taken together these data indicate that a homeostasis of ROS production is of major importance for the early infection stages and the metabolic switch leading to development of sclerotia.

PS04-193

Fumonisin B1 alters mitochondrial function and actin cytoskeleton during cell death induction in Arabidopsis

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The Fumonisin B1 (FB1), produced by Fusarium verticiloides, is a sphinganine analog mycotoxin and initiates programmed cell death (PCD) in both animals and plants. Despite the mechanisms of FB1 toxicity have been research for decades, signals and target sites during FB1-induced PCD are still largely unknown. In this work, we focused on mitochondrial behavior and signals during FB1-induced Arabidopsis cell death. By measuring and analyzing three (xyz) and four (xyzt) dimensional confocal micrographs in protoplasts and leaves, we found a dramatic increase in the size of individual mitochondrion and a concomitant decrease in the number of mitochondria per cell after FB1 treatments. FB1 induced mitochondrial oxidative burst and significant decrease of the velocity and complexity of mitochondrial movement. Further, FB1-induced mitochondrial morphological changes were highly associated with actin filaments. In addition, FB1 triggered PR2 expression and dramatic cell wall appositions with the presence of hydrogen peroxide. Our data demonstrated that the toxic mechanisms of FB1 in Arabidopsis is complex and involve several targets including cell wall, actin cytoskeleton and mitochondria.

PS04-194

Oxidative stress and amino acid balance are essential for the interaction of the plant-pathogen *Verticillium longisporum* and its host *Brassica napus*

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Verticillium longisporum is a devastating soil-borne fungal pathogen of the *Brassicaceae* family, including oilseed rape (*Brassica napus*) as the economically most important crop. Infection is initiated by hyphae from germinating microsclerotia which invade the plant vascular system through penetration of the fine roots. We investigated the reaction of the fungus to xylem sap of the host-plant by two-dimensional gel electrophoresis. Identification of 10 proteins by mass spectrometry revealed that all upregulated proteins are involved in oxidative stress response. The *V. longisporum* catalase peroxidase (CpeA) was the most upregulated protein and is encoded by two isogenes, *cpe*A-1 and *cpe*A-2 [1]. The protein expression in knockdowns of the catalase-peroxidase of *V. longisporum* were reduced by 80% and resulted in sensitivity against reactive oxygen species. *In planta*, knockdowns were inhibited in the late phase of disease development. During infection of the host plant, *Verticillium* induces the cross-pathway control to cope with imbalanced amino acid supplies [2]. Knockdowns of the transcriptional activator Cpc1 (CpcA/GCN4) were strongly reduced in pathogenicity. We suggest that oxidative stress and amino acid balance play major roles for the survival of *Verticillium* in the host-plant. (1) Singh S, Braus-Stromeyer SA, Timpner C, Valerius O, von Tiedemann A, Karlovsky P, Druebert C, Polle A, Braus GH (2012) Mol Plant Microbe Interact. 25: 569-81; (2) Singh S, Braus-Stromeyer SA, Timpner C, Tran VT, Lohaus G, Reusche M, Knuefer J, Teichmann T, von Tiedemann A, Braus GH (2010) Appl Microbiol Biotechnol. 85: 1961-76.

PS04-195

SWEET sugar transporters identified with the help of FRET sensors are highjacked for nutrition of pathogens

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The intimate association between pathogen and host is often described in terms of an " arms race ", in which strong selective pressures over time result in diverse competing mechanisms of immunity and pathogenicity. At the root of such arms races is the parasitism of fixed-carbon, water and nutrients by the pathogen. While transfer of sugars from plant to pathogen is well established, the molecular mechanisms of such transfer had remained unclear. We uncover insight into this question through identification of a novel class of sugar transporter, SWEET, using FRET sensors in a mammalian expression system. FRET nanosensors can be used to monitor sugar flux, reported as ratio change, in living cells in a minimally invasive manner. FRET sensors can be targeted to measure subcellular compartmentation. SWEETs function as sugar uptake and efflux carriers. A SWEET homolog in rice (OsSWEET11) is encoded by the resistance locus *XA13*, which is a susceptibility factor for *Xanthomonas oryzae* infection (1, 2). Both OsSWEET11/Xa13/Os8N3 and OsSWEET14/Os11N3 are co-opted by *X. oryzae*; specific effectors secreted by *X. oryzae* can bind to specific SWEET promoters and activate transcription (1, 3). Interestingly, different pathogens induce different SWEETs in Arabidopsis, indicating that many pathogens depend on SWEET activity. The identification of SWEETs as susceptibility factors opens new perspectives on the role of bacterial effectors and provides new tools to study plant pathogen interactions. (1) Chu Z. *et al*. *Gene Dev.* 20, 1250-1255 (2006); (2) Yang B.*et al*. *PNAS* 103, 10503-10508 (2006); (3) Antony G.*et al*. *Plant cell* 22, 3863 (2010).

PS04-196

***Magnaporthe oryzae* evades MAMP-triggered immunity of the host plants with surface-accumulated α-1,3-glucan on the cell wall**

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Plants evoke innate immune defenses against fungal challenges upon recognition of MAMPs such as chitin, a major cell wall component of fungi. Nevertheless, fungal pathogens somehow circumvent the innate immunity of host plants. We previously reported that the rice blast fungus *Magnaporthe oryzae* masks cell wall surface with α-1,3-glucan, an undegradable polysaccharides for many plants, in response to a plant wax component via activation of Mps1 MAPK signaling (Fujikawa et al., 2009). We further elucidated role of α-1,3-glucan in *M. oryzae*-rice interactions. A *M. oryzae* mutant lacking α-1,3-glucan normally produced infectious structures. However, the inoculation of the mutant rapidly induced defense responses in the susceptible rice plants and, as a result, the fungal infection was completely blocked. Moreover, a transgenic rice expressing a bacterial α-1,3-glucanase showed strong resistance to the fungal infection. Overall, our results suggest that the surface α-1,3-glucan plays indispensable roles in escaping the host innate immunity in *M. oryzae*. We will discuss role of α-1,3-glucan in innate immune evasion mechanisms in fungal plant pathogens and potentiality of novel plant protection approaches that targets fungal α-1,3-glucan.

PS04-197

Enhancement of chitin elicitor responses by engineering the chitin elicitor receptor CEBiP improves disease resistance against rice blast fungus

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Chitin oligosaccharides are derived from fungal cell walls and elicit various immune responses in plants, which contributes to innate immunity against fungal diseases. The chitin elicitor (CE) is recognized by receptors localized to the plasma membrane, thus, enhancement of CE-triggered responses by engineering the CE receptor is expected to bring about more fungal disease tolerance to plants. To enhance CE-triggered responses in rice, we constructed two types of chimeric receptors using rice CE receptor, CEBiP, and receptor-like protein kinases in rice. CRXAs are fusion proteins between CEBiP and the intracellular region of Xa21, which confers resistance to rice bacterial blight, connected with a transmembrane domain (TM). CRPis are fusion proteins between CEBiP and the intracellular region of Pi-d2, a true resistance gene against *Magnaporthe oryzae* carrying *AvrPi-d2*. Transgenic rice expressing *CRXA* showed increased cellular responses to CE (e.g. ROS generation, RNS generation, and cell death induction) and more tolerance to *M. oryzae*. Transgenic rice expressing *CRPi* also showed increased cellular responses to CE and more tolerance to *M. oryzae*, but those phenotypes depended on the TM structure used. The intracellular protein kinase region of the chimeric receptors was required for the enhanced responses to CE and disease resistance. These results strongly suggest that the CEBiP-based chimeric receptors act as functional receptors for CE in rice cells and fungal disease resistance is improved by the enhancement of CE-triggered responses.

PS04-198

Molecular and genetic approaches to explore the melon-Fusarium interaction

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Four races of *Fusarium oxysporum* f.sp. *melonis* (FOM) exist, that cause severe damage to melon (*Cucumis melo* L.) worldwide. A whole range of interactions, from fully susceptible to tolerant, quantitatively resistant and fully resistant, have been described between different melon genotypes and specific FOM races. Dominant monogenic resistances against FOM races 0, 1 and 2 have been mapped. Of these, the *Fom-2* gene has been cloned, and a transgenic root system ("Composite Plants") served to examine R-gene promoter and protein function in melon roots. Regarding FOM race 1.2, a quantitative mode of inheritance was proposed, and we have characterized it as a recessive trait, controlled by two major recessive genes, when severe artificial inoculation is applied, but in the field it appeared as a dominant trait. A mapping population that segregates for FOM1.2 resistance was used for QTL analysis. The infection process of a FOM 1.2 strain that expresses the GFP reporter protein was monitored *in vivo* in infected roots and stems, indicating the time points and sites in which fungal progression differed between resistant and susceptible genotypes. Constitutive and induced expression patterns of defense genes were compared between resistant and susceptible genotypes, using real-time PCR. Both the constitutive and inducible defense responses could contribute to the reduced vascular colonization of the resistant genotype.

PS04-199

Identification and functional characterization of *Phytophthora infestans* RXLR effectors suppressing flg22-triggered early signalling in both *Arabidopsis* and Tomato

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The genome of *Phytophthora infestans*, the causal agent of potato and tomato late blight, is encoding several hundreds of so-called RXLR effectors which are thought to be translocated inside the host cells during infection (1). In order to elucidate the biological function of RXLR effectors (PiRXLRs) *in planta*, we used a protoplast-based system to assess their potential for subverting plant immunity by manipulating MAMP-triggered early signalling pathways (2, 3). Fourty-five PiRXLR effectors were tested for their ability to suppress the activation by flg22 of a reporter gene under control of a typical MAMP-inducible promoter (pFRK1::Luc). Nine PiRXLR effectors blocked significantly reporter gene activation by flg22 in tomato protoplasts. Further, three of them affected post-translational MAP Kinase activation, suggesting an interference with MAMP signalling at- or upstream of the MAP kinase cascade.As MAMP-signalling pathways appear to be conserved across the plant kingdom, we hypothesized that some of our PiRXLR effector candidates may target proteins/mechanisms that are highly conserved in both host and non-host plants. From the aforementioned nine candidate PiRXLR effectors, five were confirmed to strongly inhibit flg22-induced pFRK1::Luc reporter gene activity when transiently expressed in *Arabidopsis thaliana* protoplasts. However, none of them was able to affect post-translational MAP kinase activation. Three PiRXLR effectors appeared to share similar functions in both *Arabidopsis* and tomato by suppressing transcriptional activation of MAMP-marker genes.
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PS04-200

The TritNONHOST consortium: Integrative genomic and genetic analysis of nonhost resistance across Triticeae species

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Plants are constantly exposed to potentially pathogenic micro-organisms. Each plant species, however, is a host for only a very limited number of pathogens. Whereas host plants possess variable degrees of resistance to adapted pathogens they are highly resistant to non-adapted pathogens. Therefore nonhost resistance is of significant interest for contemporary plant breeding and sustainable crop production. The aim of TritNONHOST is to join expertise in nonhost resistance in the *Triticeae* crops wheat and barley, and the economically important pathogens *Blumeria graminis*, *Puccinia spp*, and *Magnaporthe* in order to exploit nonhost-resistance for sustainable control of fungal diseases. We addressed these main questions:
- What are the commonalities and differences in gene expression between wheat and barley when comparing host and nonhost interactions? We have performed a large-scale transcript profiling experiment in six host and nonhost systems

in wheat and barley using the Agilent gene-expression arrays. - What effect do candidate genes have on the resistance phenotype in different cereal-pathogen interactions?We are performing transient and stable gene silencing experiments in both wheat and barley, targeting selected candidate genes. - Can we link the results from functional genomics to genetic loci controlling nonhost resistance? We will determine the haplotypes of all relevant candidate genes in barley and perform nonhost-QTL co-localization studies in mapping populations segregating for attenuated nonhost resistance.

PS04-201

How does vesicle-mediated exocytosis contribute to fungal defense in *Arabidopsis thaliana*?

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Plants employ multiple defense mechanisms against microbial pathogens. Previously we identified a molecular mechanism based on vesicle-mediated secretion in *Arabidopsis*. This exocytosis mechanism depends on a ternary protein complex consisting of the vesicle-resident VAMP721/722, PEN1 syntaxin, and SNAP33 (Kwon *et al*., 2008). Here we examined how this complex is regulated both in the absence and in the presence of pathogens. To reveal regulatory components, we screened for proteins that co-localize with VAMP721/722 and identified one of RAB GTPase, RABA1e. RAB GTPases are key mediators of endomembrane trafficking. Of note, RABA1a and RABA1e are transcriptionally up-regulated upon bacterial infection. We tested both RAB GTPases using T-DNA mutants for a possible role in plant defense against the host-adapted powdery mildew *Golovinomyces orontii* and the non-host fungal pathogen *Erysiphe pisi*. Both mutants reveal an altered infection phenotype not to *G. orontii*, but to *E. pisi*. Additionally, we tested the synaptotagmins (SYTs) for a possible role in immunity. The Arabidopsis *syt1* mutants, identified in a screen for salt hypersensitivity, function in plasma membrane repair. Plasma membrane integrity has not been described in biotic stress in plants. We found that*syt1* plants exhibit enhanced disease resistance not to *E. pisi* but to *G. orontii*. So far we know *Arabidopsis* has two pre-invasive resistance pathways against powdery mildew fungi that are either PEN1-dependent- or PEN2/PEN3-dependent. Therefore, we are further dissecting the role of SYT1-dependent defenses in the context of known pre-invasive disease resistance components against microbial pathogens by combined gene interaction studies and cell biological approaches.

PS04-202

Identification of an *Arabidopsis thaliana* mutant susceptible to *Botrytis cinerea* infection

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Botrytis cinerea is a necrotrophic fungus which causes severe disease in both field and postharvest situations, resulting in enormous economic losses. Seeking for resistant genes and growing disease resistant crops are effective environment-friendly strategy for protecting the crops from disease. We screened mutants from a T-DNA insertion-mutagenized Arabidopsis population. A mutant with enhanced susceptibility to *B. cinerea*, named *esb1*(enhance susceptibility botrytis1) was identified. Symptoms of infection in esb1 leaves inoculated with pathogen conidiospores appeared 2 days after inoculation, which is one day earlier than wild-type leaves. The differences in rate of disease and disease index between mutant and wild-type plants during 1-8 days after pathogen infection are significant. The trypan-blue staining revealed that dead cells appeared in mutant leaves about 24 hours after inoculation with pathogen, while no dead cell was found in wild type leaves. Further assay demonstrated that the activities of guaiacol peroxidase and catalase were no significant differences between the *esb1* and wild type plans. The content of malondialdehyde in *esb1* leaves was obviously higher than that in wild type. Additionally, the *esb1* plant displayed not only susceptibility to *Botrytis* infection, but also impaired tolerance to water deficit and increased salinity. The results indicat that *ESB1* gene probably is involved in a signal transduction of plant response to stress. The genetic analysis showed that susceptibility was inherited as a single recessive locus. TAIL-PCR assay showed that the T-DNA was inserted into the gene At4g39690. And database information reveals that the function of this particular gene remains unknown.

PS04-203

Unraveling plant regulatory networks by studying a NAC transcription factor's role towards biotic and abiotic stress

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HvNAC6 is a NAC family transcription factor gene that plays a key role in penetration resistance towards powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*) in barley (Jensen *et al*., PMB 65(1):137-150, 2007), but its underlying mechanisms are not known. Therefore we generated stable *HvNAC6* knockdown transformation lines to investigate its function in biotic and abiotic stress and regulatory mechanisms. Transgenic barley plants harbouring an *HvNAC6* RNA interference (RNAi) construct displayed lower levels of *HvNAC6* transcripts and were more susceptible to powdery mildew than wild-type plants. Moreover, *HvNAC6* knockdown plants exhibit dosage-dependent ABA hyposensitivity during seedling development, which implies *HvNAC6* modulates ABA-associated phenotypes in seedling developmental processes. Interestingly, spaying ABA on leaves before inoculation with *Bgh* reduced penetration in wild-type plants but not in *HvNAC6* knockdown plants. Another approach is to investigate the transcriptional regulation of the *HvNAC6* gene. *In silico* analysis of this promoter demonstrates the presence of similar putative regulatory elements including W box, GCC box, MYC, MYB and ABRE, which suggests that *HvNAC6* also plays an important role in abiotic stress responses. We have generated stably transformed barley plants with *HvNAC6* promoter linked to the reporter genes GUS and GFP to analyze temporal and spatial gene expression patterns occurring at a tissue and organ level following biotic and abiotic stress.

PS04-204

Peroxisomal and mitochondrial β-oxidation contributes to virulence in *Ustilago maydis*

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The biotrophic basidiomycete *Ustilago maydis* causes smut disease on corn. During infection the availability of glucose is limited and carbon sources such as lipids could be important. Filamentous growth is mandatory for pathogenicity of *U. maydis* and can be triggered by lipids. *U. maydis* posses peroxisomal, Mfe2Mfe2b, and mitochondrial, Had1Had2, β-oxidation and expression of β-oxidation genes is induced during infection. Deletion of the third step of mitochondrial β-oxidation or peroxisomal β-oxidation led to no growth on short chain fatty acids or on fatty acids >C6, respectively. Whenever mutants were unable to utilize fatty acids filamentation was also abolished. Further growth on acetate was reduced probably by interference with the glyoxylate pathway.

Short chain fatty acids induce apoptosis in *U. maydis* and blockage of β-oxidation leads to accumulation of toxic intermediates. Mating of the mutants was unaffected except for the *mfe2mfe2b* mutant. However, virulence was drastically reduced for both pathway mutants. β-oxidation could be a fungicide target because of the inability to use fatty acids, acetate and the accumulation of toxic β-oxidation intermediates. Anti-inflammatory drugs inhibit the human Mfe2. In *U. maydis* they reduce growth on fatty acids, reduce filamentation and induce apoptosis. One single dose of Diclofenac reduces the virulence of *U. maydis*. We showed the importance of β-oxidation for virulence of fungi and its potential as a fungicide target.

PS04-205

Two secretory proteins are regulated by ProA in *Epichloë festucae*, a mutualistic symbiont of perennial ryegrass

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The fungal endophyte, *Epichloë festucae*, forms a symbiotic association with perennial ryegrass, *Lolium perenne*. In wild-type associations, *E. festucae* grows systemically in the intercellular spaces of the leaves as infrequently branched hyphae parallel to the leaf axis. *proA* encodes a $Zn(II)_2Cys_6$ transcription factor that has homology to Pro1/NosA, positive regulators of sexual development in other ascomycetes. A *proA* deletion mutant causes severe stunting of the grass host, a host interaction phenotype very similar to that observed for a *noxA* deletion mutant. To identify the gene targets for ProA, we analyzed a publicly available *Sordaria macrospora* microarray data set for genes differentially expressed in a *pro1* mutant. One gene, *esdC*, significantly down-regulated in *S. macrospora pro1* mutant was also down-regulated in the *E. festucae proA* mutant/ryegrass interaction. *esdC* encodes a glycogen-binding domain protein and has been showed to be involved in sexual development in *Aspergillus nidulans*. To determine whether ProA regulates *esdC* expression by directly binding to its promoter, we prepared a fusion protein of ProA and maltose binding protein (MBP-ProA 1-120) and carried out electrophoretic mobility shift assays (EMSA). Mobility shifts were observed for two fragments of 36-bp and 38-bp from the *esdC* promoter. Analysis of the sequence upstream of *esdC* revealed the presence of divergently transcribed gene, EF320. Quantitative RT-PCR analysis revealed that the expression of EF320 was dramatically reduced in the *proA* mutant/ryegrass interaction. Analyses of the amino acid sequences of EsdC and EF320 predict signal peptides at the N-terminus of these proteins.

PS04-206

Functional genomic approaches to study nonhost resistance of *Medicago truncatula* against Asian soybean rust pathogen, *Phakopsora pachyrhizi*

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Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is a devastating foliar disease affecting soybean production worldwide. Identification of genes in another legume species that confer nonhost resistance (NHR) against ASR will provide an avenue to engineer soybean for durable resistance against ASR. We found that *Medicago truncatula*, a model legume, conferred NHR against ASR. Although the urediniospores formed germ-tube with appresorium and penetrated into epidermal cells, *P. pachyrhizi* failed to sporulate on *M. truncatula*. Transcriptome and metabolome analyses identified a role for phytoalexins and saponins during NHR response against ASR. To identify *M. truncatula* genes that confer NHR against ASR, we established a forward-genetics screen using *Tnt1* retrotransposon insertion lines and identified several mutants that show altered response upon infection with ASR. One of these mutants, *irg1* (*inhibitor of rust germ-tube differentation1*), inhibited pre-infection structure differentiation of *P. pachyrhizi* and a non-adapted switchgrass rust pathogen, *Puccinia emaculata*. Cytological and chemical analyses revealed that inhibition of rust pre-infection structures in *irg1* mutants is associated with the complete loss of the abaxial epicuticular wax crystals and surface hydrophobicity. *IRG1* encoded a Cys(2)His(2) zinc finger transcription factor, *PALM1*, that also controls dissected leaf morphology in *M. truncatula*. Transcriptome analysis further revealed down-regulation of genes involved in wax biosynthesis and secretion in the *irg1* mutant.

PS04-207

Inheritance of *Phytophthora infestans* effector-induced hypersensitive cell death in hot pepper

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Non-host resistance is most common resistance defined as a strong and durable resistance in a plant species against most potential microbial pathogens. Non-host resistance consists of various steps such as basal defense, preformed resistance, induced defense and resistance gene-mediated defense. In most cases, true resistance is mediated by interaction of resistance genes and effectors in the cytoplasm. For elucidating the molecular mechanisms of non-host resistance, 4 accessions of *Capsicum* spp. 09-11, 09-186, 09-202 and 09-226 were subjected to *in planta* interaction with 54 RXLR effectors from potato blight pathogen *Phytophthora infestans* using PVX-mediated transient expression. As results, some of effectors trigger hypersensitive response (HR)-like cell death in pepper as that of the HR in host plant. To determine the inheritance of RXLR effector-induced cell death in pepper accessions, two accessions (09-202 and 09-226) were crossed and the resulting F_1 and F_2 populations were screened against four RXLR effectors (PexRD8, PexRD24, PexRD41 and PexRD92-4). Among them, PexRD8-induced cell death in F_2 siblings of pepper accession (09-226) were segregated as 15:1. These result may indicated that two dominant genes in that accession are involved in PexRD8-induced cell death. The rest of RXLR-induced cell deaths also are shown obvious segregation ratio which have a genetic meaning. These results may suggest that non-host resistance could be correlated with the presence of multiple genes interacted specific RXLR effector facilitating durable resistance form in plants.

PS04-208

NbPDR1, a PDR-type ABC transporter, confers pre- and post-invasion resistances of *Nicotiana benthamiana* against potato late blight pathogen, *Phytophthora infestans*

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Mature *Nicotiana benthamiana* shows strong resistance to *Phytophthora infestans*. We previously showed that virus-induced gene silencing (VIGS) of *NbEAS* (5-*epi*-aristolochene synthase), encode an enzyme for the production of sesquiterpenoid phytoalexin, capsidiol, compromised the resistance. By screening using VIGS for essential genes for disease resistance, we isolated genes for expected terpenoids transporter, *NbPDR1*, and enzymes for mevalonate pathway, the upstream pathway of terpenoids

biosynthesis, including *NbMVD* (mevalonate diphosphate decarboxylase) and *NbFPPS* (farnesyl pyrophosphate synthase). Both *NbEAS*- and *NbPDR1*-silenced plants showed decreased accumulation of capsidiol, however, *NbPDR1*-silenced plant showed severer disease symptom by *P. infestans* than *NbEAS*-silenced plant. Detection of pathogen penetration sites by aniline blue staining revealed that *NbPDR1*-silenced plant impaired penetrated resistance, whereas *NbEAS*-silenced plant retained penetration resistance as control plant, indicating that *NbPDR1* is involved in the export of antifungal compounds, other than capsidiol, for penetration resistance. Production of capsidiol was decreased in both *NbMVD*- and *NbFPPS*-silenced plants, but penetration resistance was compromised only in *NbMVD*-silenced plant. Given synthesis of diterpenoids is mediated by MVD but does not require the activity of FPPS, diterpenoids, such as scrareol, was expected as compounds for the penetration resistance. Consistently, N. benthamiana with silencing of a gene for a key enzyme of diterpenoids biosynthesis, *NbGPPS* (Geranylgeranyl pyrophosphate synthase) showed significantly reduced penetration resistance. Furthermore, treatment with a diterpenoid, scrareol, suppressed the germination of zoosporangia of *P. infestans*. Altogether, these results indicated that *N. benthamiana* PDR1 contributes the export of the antifungal diterpenoids and sesquiterpenoids for pre- and post-invasion resistance against *P. infestans*.

PS04-209

Arabidopsis mutants displaying aberrant localization of the PEN3 ABC transporter have altered responses to powdery mildew fungi

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The *Arabidopsis thaliana* PEN3 ABC transporter is recruited to sites of attempted penetration by powdery mildew fungi, where it contributes to pathogen defense. Perception of pathogen-associated molecular patterns such as fungal chitin or bacterial flagellin is sufficient to initiate focal accumulation of PEN3 within the plasma membrane, suggesting that pattern recognition receptors initiate the recruitment of the transporter to sites of pathogen detection at the cell surface. Targeting of PEN3 to sites of pathogen detection requires intact actin filaments, but is not accomplished through *de novo* protein synthesis, BFA-sensitive vesicle trafficking events, or constitutive endocytic cycling. The specific mechanisms underlying polar targeting of PEN3 to sites of pathogen detection and its retention at such sites remain unclear. To identify cellular components required for proper subcellular targeting of PEN3, we performed a genetic screen for mutants displaying *aberrant localization of PEN3-GFP (alp)*. Screening of 10,000 M2 individuals by confocal microscopy yielded 9 *alp* mutants with various PEN3 localization defects, including one mutant with spontaneous polar targeting of PEN3 in the absence of pathogen stimulus. Initial characterization has revealed that several mutants have altered responses to powdery mildew fungal infection. Mapping and further characterization of alp mutants is currently underway.

PS04-210

Screening for candidates of *PWT4*, a gene for avirulence of an *Avena* isolate of *Magnaporthe oryzae* on wheat, using whole-genome sequencing

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Pwt4 is a locus conditioning the avirulence of an *Avena* isolate of *Magnaporthe oryzae* on wheat. *Avena* isolate Br58 carries the avirulence allele *PWT4* while *Triticum* isolate Br48 carries the virulence allele *pwt4*. Here, we report the identification of *PWT4* candidates by the whole-genome sequencing of pooled DNA from a *PWT4*-segregating population. An F_1 culture carrying *PWT4* was chosen from an F_1 population derived from a genetic cross between Br58 and Br48, and backcrossed with Br48 four times to produce a BC_4F_1 population in which *PWT4* segregated. DNAs were extracted from 8 BC_1F_1 and 40 BC_4F_1 cultures carrying *PWT4*, pooled, and seqeuenced. By aligning the resulting sequence reads on the genomic sequence of Br58, we could identify a 580kb genomic region of Br58 shared by the *PWT4* carriers. Genes with a secretion signal in the 580kb genomic region were predicted by using gene prediction software fgenesh and signal peptide prediction software signalP. Then, predicted genes were further screened based on the polymorphisms between the two parental isolates. Consequently, 3 genes encoding putative secreted proteins were selected as *PWT4* candidates. Among the 139 genes predicted by fgenesh, more than 60% (86 genes) were present in the genome of Br58 but not in Br48, suggesting that *PWT4* is located on or around a Br58-specific genomic region.

PS04-211

Involvement of S-nitrosylated StRanBP1 in plant defense response

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Nitric oxide (NO) has various functions in physiological responses of plant, such as development, hormone signaling, and defense. The mechanism of how NO regulates physiological responses has not been understood well. Protein S-nitrosylation, a redox-related modification of cysteine thiol by NO, is known as one of the important post translational modification to regulate activity and interactions of proteins. By using biotin switch method, changes in S-nitrosylated proteins in potato (*Solanum tuberosum*) challenged with *Phytophthora infestans* were detected. From the proteomic approach, approximately 80 S-nitrosylated candidate proteins were identified in potato leaves and tuber treated with NO donor. Small GTPase Ran binding protein (StRanBP1) was identified as S-nitrosylated candidate protein. RanBP1-silenced *Nicotiana benthamiana* plant showed stunted growth and constantly expressed defense-related genes. To analyze function of RanBP1, StRanBP1 were fused to HA tag and expressed transiently in *N. benthamiana* leaves by *Agrobacterium* infiltration. Expression of StRanBP1 in leaves caused cell death under the dark condition and impaired the resistance against *P. infestans*. Moreover, induction of cell death and reduction of resistance by StRanBP1 expression were not observed when a mutation in expected S-nitrosylated 127th cysteine (C127A) was introduced in StRanBP1. In addition, StRanBP1 was S-nitrosylated in *N. benthamiana* leaves inoculated with *P. infestans*. Thus, redox-mediated S-nitrosylation of RanBP1 would have an important role in the defense response.

PS04-212

Evolutionary and working models for the coupled genes of the *Pik-h* encoding blast resistance of rice

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Pik-h, which is an allele of *Pik*, confers resistance against certain races of rice blast. Its positional cloning showed that it comprises a pair of NBS-LRR genes, *Pikh-1* and *Pikh-2*. The allele is distinguishable from other known blast resistance genes on the basis of key variable nucleotides, and SNP diagnosis among the five rice populations implies that it appears to be the most recently evolved of the set of *Pik* alleles. Comparisons between the sequences of *Pik-h* and other *Pik* alleles showed that the functional K haplotype exists as two sub-haplotypes, which both

evolved prior to the domestication of rice. While *Pikh-1* appears to be constitutively transcribed, the transcript abundance of *Pikh-2* responds to pathogen challenge, suggesting that while Pikh-1 may well be involved in elicitor recognition, Pikh-2 is more likely to be responsible for downstream signalling. In vitro, the CC domain of Pikh-1 was shown interact directly with both AvrPik-h and Pikh-2. Transient expression assays demonstrated that Pikh-2 mediates the initiation of the defence response. In the proposed *Pik-h* resistance pathway, it is suggested that Pikh-1 acts as an adaptor between AvrPik-h and Pikh-2, while Pikh-2 transduces the signal to trigger *Pik-h*-specific resistance.

PS04-213

Sequencing and analysis of the Pi50(t), a novel broad-spectrum resistance genes in rice

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Rice blast, caused by Magnaporthe oryzae, is one of the devastating rice diseases in the worldwide, and excavation and utilization of broad-spectrum resistance genes is an important avenue to control this disease. We have identified a broad-spectrum blast resistance gene Pi50(t), a new member of Pi2/9 multigene family, from a resistance donor Er-ba-zhan (EBZ) in South China. To identify the candidate genes of Pi50(t) locus, we sequencing the Pi2/Pi9 locus of EBZ by using the genomic walking sequencing strategy. Annotation of the sequences indicated that there are 7 NBS type R gene candidates were located in the mapping region of Pi50(t) locus, and the deduced amino acid sequence identity of these 7 R genes ranged from 75% to 100% compared with the listed Oryza sativa NBS R genes from Genbank. We further revealed that the resistance spectrum and race specificity of Pi50(t) allele were different from the other known Pi2/Pi9 carriers. For inquiring the molecular basis of broad-spectrum resistance, the screening of the key variation of Pi50(t)-NBS R genes and its multiple alleles by target region re-sequencing were performed. Cladistic analysis based on the protein sequence of these Pi2/9 NBS paralogous genes further revealed that 2 of Pi50-NBS are grouped into the same phylogenetic clade which contain Pi2 and Pi9. However, they shared a very low similarity, indicated the variation of them are very high. May thus indicate that these 2 specific NBS R genes in the Pi50(t) locus were the main functional candidates in EBZ rice cultivar.

PS04-214

Identification of a novel Fusarium wilt-resistance protein from tomato

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Fusarium wilt disease in tomato is the result of vascular tissue colonisation by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). The resistance genes *I*, *I-2* and *I-3* have been incorporated into cultivated tomato (*Solanum lycopersicum*) from wild tomato species to confer resistance against *Fol* races 1, 2 and 3, respectively. Work towards the isolation of *I-3* over a number of years has discovered *I-3* to be a member of an S receptor-like kinase (SRLK) gene family. Three closely related candidate SRLK genes were tested to determine the identity of *I-3*. One of these genes has been shown to confer full resistance to *Fol* race 3 while a second gene confers partial resistance. Two pathogen-derived proteins have been found to influence *I-3* resistance, either activating (Avr3) or suppressing (Avr1) resistance. Both are small disulphide-bonded proteins secreted into the xylem sap during infection where they could interact with the membrane bound I-3. Interestingly, Avr1 also suppresses *I-2* resistance which is conferred by a cytoplasmic NB-LRR protein. The identification of *I-3* as a new type of plant resistance protein suggests that a novel signaling pathway and downstream response genes have been recruited in the defence against *Fol*. To investigate this possibility, we have used Illumina sequencing to compare the transcription profiles of resistant and susceptible tomato challenged with *Fol* race 3 and uninfected tomato. Analysis of this this data is currently underway.

PS04-215

***PEN* and jasmonic acid mediate resistance in *Arabidopsis* against *Alternaria alternata* infection**

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The tomato pathotype of *Alternaria alternata* causes Alternaria stem canker on tomato depending upon the production of the host-specific AAL-toxin. Defense mechanisms in host and nonhost plant to *A. alternata*, however, are largely unknown. The objective of this study is to elucidate the mechanisms of nonhost resistance to toxin-dependent necrotrophic pathogen *A. alternata* using *Arabidopsis* mutants. *Arabidopsis* ecotype Columbia was insensitive to AAL-toxin and showed either no symptoms or a hypersensitive reaction (HR) when inoculated with *A. alternata*. Yet, when the *Arabidopsis penetration* (*pen*) mutants, *pen2-1*, *pen2-2* and *pen3-1* which were identified as factors of pre-invasion resistance against nonadapted powdery mildew pathogens, were challenged with *A. alternata*, fungal penetration was evident and HR-like cell death concomitant with H_2O_2 accumulation and callose deposition occurred at the site of attempted fungal invasion. However, conidiation were limited on these mutants. Meanwhile, AAL-toxin-producing *A. alternata* could invade *loh2* mutant, which have a defect in the toxin resistance gene, subsequently allowing the fungus to complete its life cycle. Jasmonic acid (JA) signaling marker gene expression was enhanced in *pen* mutants and decreased in *loh2* mutant during fungal infection. Moreover, disease symptoms were increased in double mutant combinations of *pen2* with JA signaling. These results indicate that the nonhost resistance of *Arabidopsis* to *A. alternata* consists of sequential defense systems that include pre-invasion resistance via *PEN2* and *PEN3* and JA signaling-dependent post-invasion resistance. These findings suggest that toxin-dependent necrotrophic *A. alternata* is required to overcome multilayered defense mechanisms to establish a full compatible interaction on plants.

PS04-216

A nuclear pore complex protein, Nup75, is involved in ethylene biosynthesis for phytoalexin production of *Nicotiana benthamiana* in the defense responses against *P. infestans*

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Mature *Nicotiana benthamiana* shows strong resistance to potato late blight pathogen *Phytophthora infestans*. By screening using virus-induced gene silencing, we isolated a gene for a nuclear pore complex protein, Nup75 (nucleoporin 75), as a required gene for disease resistance of *N. benthamiana* against *P. infestans*. *NbNup75*-silenced plant showed no detectable growth defect, but resistance to *P. infestans* was significantly compromised. Defense responses of *NbNup75*-silenced *N. benthamiana* induced by treatment with INF1, an elicitor protein derived from *P. infestans*, such as production of reactive oxygen species, induction of hypersensitive reaction-like cell death and accumulation of phytoalexin were suppressed as

compared to control non-silenced plant. Previously, we reported that expression of genes for phytoalexin biosynthesis, *NbEAS* (5-epi-aristolochene synthase) and *NbEAH* (5-epi-aristolochene 1,3-dihydroxylase), were induced by treatment of ethylene, and INF1-induced expression of *NbEAS* was suppressed by silencing of *NbEIN2*, a gene for ethylene signaling. Production of phytoalexin induced by INF1 treatment was impaired in *NbEIN2*-silenced plant, indicating that phytoalexin biosynthesis of *N. benthamiana* is regulated via ethylene signaling. In *NbNup75*-silenced plant, induction of *NbEAS* expression by ethylene treatment was comparable to non-silenced plant, whereas ethylene biosynthesis induced by INF1 treatment was markedly reduced. Additionally, the expression of a gene for ethylene biosynthesis, *NbACS* (1-aminocyclopropane-1-carboxylate synthase) was significantly decreased in *Nup75*-silenced plant as compared to control plant. Collectively, these results suggest that Nup75 is involved in the transcriptional up-regulation of *NbACS* for ethylene biosynthesis, which is essential for the phytoalexin production of *N. benthamiana* during the defense response against *P. infestans*.

PS04-217

Large-scale gene disruption in *Magnaporthe oryzae* identifies MC69, a secreted protein required for infection by monocot and dicot fungal pathogens

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To search for virulence effector genes of the rice blast fungus, *Magnaporthe oryzae*, we carried out a large-scale targeted disruption of genes for 78 putative secreted proteins that are expressed during the early stages of infection of *M. oryzae*. Disruption of the majority of genes did not affect growth, conidiation, or pathogenicity of *M. oryzae*. One exception was the gene *MC69*. The *mc69* mutant showed a severe reduction in blast symptoms on rice and barley, indicating the importance of MC69 for pathogenicity of *M. oryzae*. The *mc69* mutant did not exhibit changes in saprophytic growth and conidiation. Microscopic analysis of infection behavior in the *mc69* mutant revealed that MC69 is dispensable for appressorium formation. However, *mc69* mutant failed to develop invasive hyphae after appressorium formation in rice leaf sheath, indicating a critical role of MC69 in interaction with host plants. *MC69* encodes a hypothetical 54 amino acids protein with a signal peptide. Live-cell imaging suggested that fluorescently labeled MC69 was not translocated into rice cytoplasm. Site-directed mutagenesis of two conserved cysteine residues in the mature MC69 impaired function of MC69 without affecting its secretion, suggesting the importance of the disulfide bond in MC69 pathogenicity function. Furthermore, deletion of the *MC69* orthologous gene reduced pathogenicity of the cucumber anthracnose fungus *Colletotrichum orbiculare* on both cucumber and *Nicotiana benthamiana* leaves. We conclude that MC69 is a secreted pathogenicity protein commonly required for infection of two different plant pathogenic fungi, *M. oryzae* and *C. orbiculare* pathogenic on monocot and dicot plants, respectively.

PS04-218

Biochemical analysis of a *Magnaporthe oryzae* avirulence factor, AVR-Pii

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We have previously isolated AVR-Pii from *Magnaporthee oryzae* (Yoshida et al. 2009). Currently we are studying effector function of AVR-Pii. Live cell imaging showed that AVR-Pii is translocated to inside of rice cells during early infection stage of *M. oryzae*. When AVR-Pii was expressed in rice cells, it accumulated in soluble fraction of cell lysate, and gel filtration analysis showed that AVR-Pii formed two different complexes in the lysate. Based on the results of gel filtration and co-immunoprecipitation, it was suggested that one form of the complexes was homo-multimer of AVR-Pii, and the other was AVR-Pii-host protein complex. Immunoprecipitaion assay and mass-spectrometry analysis identified two rice Exo70 proteins (OsExo70-1 and OsExo70-2) as candidate interactors of AVR-Pii in rice cells. Exo70 is known as a member of exocyst complex regulating exocytosis pathway in yeast and mammals. In rice, more than 40 members of OsExo70 family are known, although only two of them, OsExo70-1 and OsExo70-2, were identified in this study. We hypothesize that AVR-Pii specifically targets OsExo70-1 and OsExo70-2.

PS04-219

Identification of novel non-host resistance genes in the Arabidopsis soybean rust interaction

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The causal agent of Asian soybean rust (ASR), *Phakopsora pachyrhizi*, represents one of the most important pathogens of soybean and other leguminous plants. Until now, control of the pest can only be achieved by expensive fungicide treatments. Since commercially available soybean varieties with stable resistance to different isolates of *P. pachyrhizi* are not yet available, new strategies are needed to counteract ASR spread and establishment. We aim at elucidating the molecular basis of Arabidopsis' non-host resistance to ASR to exploit these resistance traits for engineering of durably resistant soybean varieties. Employing a global gene expression approach we have identified genes which putatively antagonize the establishment of fungal haustoria in infected plant tissue. By applying dsRNAi-mediated gene silencing, we have analyzed most of these candidate genes with respect to their function in the Arabidopsis ASR interaction. In a complementary approach these genes have been stably overexpressed in soybean. Here, we present genes which are contributing to Arabidopsis' postpenetration resistance to *P. pachyrhizi* and/or which are capable of enhancing soybean resistance to the rust fungus.

PS04-220

Molecular cloning and analysis of a gene family encoding xylanase in *Phytophthora parasitica*

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Phytophthora parasitica is an oomyceteous plant pathogen with a wide host range. To investigate the role of genes encoding xylanase in this pathogen, we cloned 4 genes encoding endo-β-1,4-xylanase from *P. parasitica*, named *ppxyn1*, *ppxyn2*, *ppxyn3*, and *ppxyn4*, respectively. Analysis of the deduced amino acid sequences indicated that all these genes contain a signal peptide at the N-terminus and an active site signature of xylanase. Moreover, all of them belong to glycosyl hydrolase family 10. Phylogenetic analysis revealed that *ppxyn* genes form a cluster which is distinct from xylanase genes of fungal pathogens. Heterologous expression of recombinant proteins by *Pichia pastoris* followed by reducing sugar assay confirmed that each of these proteins have xylanase activity toward brichwood xylan. Analysis by quantitative reverse transcriptase-PCR demonstrated *ppxyn1* and *ppxyn2* were up-regulated at the early stage of *P. parasitica* infection on tomato leaves. The expression of *ppxyn3* and *ppxyn4*, in contrast, were

detected predominantly at the stage of cyst and germinated cyst of *P. parasitica*, but only slightly induced in the infection process. To investigate their role in the pathogenicity of *P. parasitica*, we silenced the expression of *ppxyn1* and *ppxyn2* in *P. parasitica* and performed inoculation experiments on *Nicotiana benthamiana*. It was found that the transformants, which showed reduced expression of *ppxyn1* and *ppxyn2*, could still infect plants, although the resulting disease symptom was only slightly severe compared to that caused by the wild-type strain.

PS04-221

Formation of highly branched hyphae by *Colletotrichum acutatum* within the fruit cuticles of *Capsicum* spp.

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Plant pathogenic fungi have evolved sophisticated strategies to penetrate plant cell walls. *Colletotrichum* species are well known for their ability to penetrate the host cuticle with penetration pegs. This study reports that *C. acutatum* penetrates the cuticle layer of *Capsicum* spp. fruits by forming a previously uncharacterized structure from appressoria. This unusual structure was localized in the cuticle layer. The structure, formed within 24 h post-inoculation (hpi), is highly branched, well-differentiated hypha which penetrates into epidermal cell at 72 hpi. The novel structure, composed of abnormally thick walls (about 250 nm), often formed multiple branches in the affected chili pepper. This dendroid structure, likely required for penetration, was formed exclusively in the cuticle layer of chili pepper fruits and not found when *C. acutatum* was inoculated on pepper petals, mango leaves, or fruits of tomato and eggplant. *C. acutatum* produced similar dendroid structure within the resistant chili pepper fruit but eventually the structure turned into dark brown and no further infection in the epidermal cell occurred, implicating the presence of inhibitors for the formation and development of the dendroid penetration structure in the resistant line. Taken together, the results indicate that a unique structure was formed by *C. acutatum* during the penetration of chili pepper fruit.

PS04-222

Functional analysis of an oxidative stress-regulated gene *MfAP1* from *Monilinia fructicola*

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Redox control in fungal development and pathogenicity is an emerging area of research in plant-microbe interactions. The fungal pathogen *Monilinia fructicola* causes brown rot blossom blight and fruit rot in many species of *Prunus* and remains quiescent on stage II fruit, which contain high levels of chlorogenic acid, a quinate ester of caffeic acid (CA). Our previous study has shown that the influence of CA on *M. fructicola* virulence is related to redox status and the yeast AP-1-like transcription factor (YAP1) may be involved in the redox regulation. YAP1 is activated under oxidative stress and has a crucial role in fungal development and pathogenicity in many fungal pathogens. We have cloned a *YAP1* gene from *M. fructicola* (*MfAP1*) and have found that *MfAP1* expression is regulated by CA and H_2O_2. To investigate the function of the *MfAP1* in the development and pathogenicity of *M. fructicola*, *MfAP1* silenced strains were created using a silencing vector pSilent- Dual1 carrying a 500-bp *MfAP1* fragment. Four *MfAP1* silenced strains were obtained. These *MfAP1* silenced mutants displayed higher sensitivity to H_2O_2 than wild type strain. Fungal pathogenicity assay reveals that *MfAP1* is required for the full virulence of *M. fructicola*. However, these *MfAP1* silenced strains were heterokaryotic and are unable to maintain the silencing construct in their genome consistently. Therefore, functional analyses of *MfAP1* using *MfAP1* overexpression strains are under way and the results will be presented and discussed.

PS04-223

Induction and regulation of highly branched penetration structure of *Colletotrichum acutatum*

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Chili pepper anthracnose caused by *Colletotrichum acutatum* results in severe losses in yield and fruit quality in Taiwan. Our previous study (Liao et al., 2011, Plant Pathology) has shown that *C. acutatum* penetrates the cuticle layer of *Capsicum* spp. fruits by forming a highly branched penetration structure (HBPS) which was not previously characterized in any *Colletotrichum*-plant interactions. Here we reported the results on the induction and regulation of HBPS. Pepper fruits cuticle layer PC1, PC2, PC3 and PC4 were isolated. *C. acuttaum* forms the highest frequency of HBPS in PC2. Therefore, PC2 was used to study HBPS induction and regulation. Environmental factors have been shown to regulate hyphal branching in *Neurospora crassa*. Our Data showed that HBPS formation can be regulated by light, temperature and osmotic stress. Light has highly significant impact on HBPS formation, the longer exposure time the lower proportion of HBPS formation. Signal transduction pathways which may be involved in HBPS formation, including cAMP dependent protein kinasen A, calicium/calmodulin, MAP kinase pathway, were studied using pharmacological effectors. HBPS formation was found to be inhibited by the stimulators of cAMP dependent protein kinase A and by the inhibitors of phospholipase C and MAP kinase. The imbalance of cellular calcium level seems also have a role on HBPS formation.

PS04-224

Heterochromatic marks regulate secondary metabolite biosynthesis in *Epichloe festucae* and the symbiotic interaction of this fungal endophyte with perennial ryegrass

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The fungal endophyte *Epichloe festucae* systemically colonizes perennial ryegrass (*Lolium perenne*), and produces a range of secondary metabolites, including lolitrems, that protect the host from mammalian herbivory. We have shown that the ten *ltm* genes required for lolitrem biosynthesis are not expressed in culture but highly expressed *in planta*. Recent work showed that disruption of genes encoding either heterochromatin protein-1 (HepA) or the H3K9 methyltransferase (ClrD) in *Aspergillus nidulans* resulted in enhanced expression of secondary metabolite gene clusters, demonstrating that heterochromatic marks are involved in the repression of these clusters. Thus, we propose that the three closely linked *E. festucae ltm* gene clusters have a repressive chromatin structure in culture, and chromatin remodeling is required for activation *in planta*. To test this hypothesis we have deleted the *hepA* and *clrD* homologues from *E. festucae* by targeted gene replacement. Deletion of *hepA* resulted in a slight reduction in culture radial growth whereas deletion of *clrD* resulted in a severe reduction. Expression levels of *ltmM* (cluster 1) and *ltmP* (cluster 2), as measured by qRT-PCR, increased in the *δhepA* mutant grown in a defined medium. In addition, the *δhepA* mutant has a dramatic host interaction phenotype, inducing severe stunting and premature senescence of the ryegrass host. Introduction of a wild-type allele of *hepA* complemented both *δhepA* mutant phenotypes. These results suggest that heterochromatic marks regulate both lolitrem gene expression and the mutualistic symbiotic interaction of *E. festucae* with its host perennial ryegrass. Phenotype analysis of the *δclrD* mutant is in progress.

PS04-225

The direct protein-protein interaction results in the arms race co-evolution between *Magnaporthe oryzae AVR-Pik* and rice *Pik*

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Between pathogen and host, antagonistic interactions impose strong reciprocal selection on each organism, leading to the development of arms race evolutionary dynamics. However, studies on specific recognition and co-evolution between resistance (R-) gene and avirulence (AVR-) gene are still limited. Here we show that *AVR-Pik* of *Magnaporthe oryzae*, the rice blast pathogen, and cognate rice R-gene *Pik* exhibit high levels of DNA polymorphisms causing amino acid changes. We found a tight recognition specificity of *AVR-Pik* alleles by different *Pik* alleles. Pik is composed of two kinds of CC-NBS-LRR, Pik1 and Pik2. We found that AVR-Pik physically interacts with the N-terminal coiled-coil domain of Pik1 in yeast 2-hybrid assay as well as in *in-planta* co-immunoprecipitation assay. Furthermore, this binding specificity corresponds to the recognition specificity between AVR-Pik and Pik alleles. These data suggest that the direct protein-protein interaction results in the arms race co-evolution between *AVR-Pik* and *Pik*.

PS04-226

Arabidopsis WRKY18- and WRKY40-regulated host responses in plant immunity

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Transcriptional reprogramming represents a vital component of the overall host defense machinery triggered in response to phytopathogen challenge. Recently, we showed that simultaneous mutation of two WRKY-type transcription factors, WRKY18 and WRKY40, rendered otherwise susceptible wild type *Arabidopsis* plants resistant towards the biotrophic powdery mildew pathogen *Golovinomyces orontii*. This resistance was accompanied by an imbalance in JA/SA signaling, exaggerated expression of certain defense genes, and elevated camalexin levels (Pandey et al., TPJ 64, 912, 2010). Our current studies are focused on determining the signaling pathways in which WRKY18 and WRKY40 act, and in identifying direct targets of these two transcription factors. Data will be presented showing that SA is essential for resistance towards *G. orontii* in the *wrky18 wrky40* background but that additional biochemical pathways are also required. Moreover, whereas WRKY18 and WRKY40 act as negative regulators of basal defense towards *G. orontii* this is not the case for other tested powdery mildews. Thus, their loss-of-functions do not confer broad-spectrum resistance towards these powdery mildew fungi. Interestingly, WRKY18 and WRKY40 also act as positive regulators of RPS4-mediated resistance as *wrky18 wrky40* double mutants were found to be strongly susceptible towards *Pseudomonas syringae* DC3000 bacteria expressing the *avrRPS4* effector gene. This response appears to be highly specific since it was not observed with bacteria expressing other *avr* genes.

PS04-227

Necrosis and ethylene-inducing peptide-like proteins of the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis*; Contradictio in Terminis?

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The obligate biotrophic pathogen *Hyaloperonospora arabidopsidis* expresses several Necrosis and ethylene-inducing peptide (Nep1)-Like Proteins (NLPs) during infection of *Arabidopsis*. In the *H. arabidopsidis* genome, we found that 12 of a total of 14 *NLP* genes form a species-specific cluster when compared with other oomycete *NLP* genes, suggesting this class of effectors has recently expanded. As NLPs are best known for their phytotoxicity it is surprising that this obligate biotrophic pathogen has an expanded *NLP* gene family. Contrary to most of the studied *NLP* genes, none of the *HaNLP*s causes necrosis when expressed *in planta*. Even HaNLP3, which is most similar to necrosis-inducing NLP proteins of other oomycetes and which contains all amino acids that are critical for necrosis-inducing activity, did not induce necrosis. Chimeras constructed between HaNLP3 and the necrosis-inducing PsojNIP protein demonstrated that most of the HaNLP3 protein is functionally equivalent to PsojNIP, except for an exposed domain that prevents the induction of necrosis. The early expression and species-specific expansion of the *HaNLP* genes is suggestive of an alternative function of noncytolytic NLP proteins during biotrophic infection of plants. We will report on our advances in analyzing *Arabidopsis* lines expressing different *HaNLP*s. As the *Arabidopsis* plants constutitively expressing *HaNLP*s show a severe phenotype, we have also created inducible *HaNLP3* lines to study the effects of these proteins on host cell processes.

PS04-228

***Verticillium* manipulates RNA silencing to suppress host immunity**

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RNA silencing is the regulation of gene expression based on the accumulation of sequence-specific small RNAs (sRNAs) that target messenger RNAs (mRNAs) resulting in their degradation. Several genes controlling RNA silencing in plants have been identified. The plant RNA silencing pathway mediates plant immunity against viruses and bacteria.Previous data from our laboratory indicate that fungus *Verticillium dahliae* also targets the plant RNA silencing pathway, presumably by secreted effectors, to suppress host defence (1). How *Verticillium* manipulates the RNA silencing pathway to suppress host immunity is still unknown. We are using the model plant Arabidopsis that is a host of *Verticillium* to unravel the role of RNA silencing in *Verticillium* wilt disease. We plan to identify the secreted *Verticillium* effectors and the Arabidopsis components that play a role in RNA silencing and are essential for *Verticillium* wilt disease. We are currently identifying *Verticillium* regulated mRNAs and sRNAs of the host, and *Verticillium* effectors that target host RNA silencing by combining transcriptomics, sRNA profiling, and effector screening. The obtained results will be presented. (1) Ellendorff U, Fradin EF, de Jonge R, Thomma BP. (2009) RNA silencing is required for Arabidopsis defence against Verticillium wilt disease. *J Exp Bot.*; 60 (2):591-602.

PS04-229

COM1* encodes a novel component of the spliceosome to regulate conidium development and virulence in *Magnaporthe oryzae

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Rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases of rice worldwide. The rice blast fungus produces pyriform conidia as the primary inocula and the main source for dissemination in the field. We previously identified a novel gene *COM1* that is required for maintaining the conidium morphology and full virulence of the rice blast fungus. We here show that *COM1* encodes a novel component of the spliceosome. Com1 is a nuclear protein containing two C-terminal regions, one lysine-proline-rich region and two nuclear localization signals that are required for functions. With the pull-down technique, 49 nuclear proteins were identified to co-immunoprecipitate with Com1-3FLAG fusion. Twenty-five of the proteins showed highest similarity to components of the spliceosome. Notably, Com1 directly interact with three Sm snRNP proteins, and the C-terminal regions were essential for the interactions. Transcriptome comparison showed that alternative splicings of pre-mRNAs for hundreds of genes was impaired in the *δcom1* mutant. Several of the genes were demonstrated to be important for conidiogenesis, the conidium morphology and plant infection. Similarly, *FgCOM1*, the *COM1* ortholog in *Fusarium graminearum* is also required for the normal conidium morphology and full virulence toward wheat and could rescue the defects of the *δcom1* mutant. These results thus indicate that Com1 and its orthologs in filamentous ascomycetous fungi are an important component of the spliceosome for accurate splicing.

PS04-230

AT-box as a novel *cis*-element for a bHLH protein and a JAZ protein to regulate expression of rice defense genes

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Identification of *cis*-elements in a gene promoter and their corresponding binding proteins contributes to understanding regulatory mechanisms of gene expression. We isolated a rice gene named *OsPinA*, which could be induced in rice in response to the infection of *Magnaporthe oryzae* and exogenous salicylic acid or jasmonic acid. Multiple *cis*-elements in the gene promoter were identified to be important for the response to the biotic and abiotic stimuli, including a 50-bp fragment, which was positively involved in the induction. With the one hybrid screening using this 50-bp fragment as bait, two proteins, named OsbHLH140 and OsJAZ11, respectively, were isolated and confirmed to be able to bind to the fragment. Deletion analysis revealed a 10-bp motif in the fragment designated as AT-box that was essential for the binding. Bioinformatics analysis and qRT-PCR assays revealed that 118 rice genes have the AT-box within 1-kb upstream of the protein translation start site and most of them could be induced together with OsbHLH140 by infection of the rice blast fungus. Furthermore, OsbHLH140 and OsJAZ11 were demonstrated to be nuclear proteins, and could interact with each other in yeast and tobacco cells. Besides, OsbHLH140 was confirmed to have transcription activation activity. These results indicate that the AT-box is a novel *cis*-element for OsbHLH140 and OsJAZ11 to regulate expression of rice defense genes.

PS04-231

MoPacC acts as a transcription repressor and an activator in *Magnaporthe oryzae* via distinct processed forms

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PacC pathway named after the PacC transcription factor is a conserved pH signaling pathway that allows fungi to survive in different pH environment. In this study, we show that deletion of MoPacC, the PacC ortholog of Magnaporthe oryzae, resulted in compact and darker colony, less conidiation and less virulent, notably arrested biotrophic growth. MoPacC exists *in vivo* in four forms, MoPacC559, the full-length form with three truncated forms, $MoPacC^{266}$, $MoPacC^{222}$ and $MoPacC^{80}$. Under acidic and neutral pH, MoPacC exists mainly as $MoPacC^{559}$ and $MoPacC^{80}$ that were localized in cytoplasm. In contrast, under alkaline pH, $MoPacC^{266}$ and $MoPacC^{222}$ occurred and localized in nuclei with some amount of $MoPacC^{559}$ and $MoPacC^{80}$. Except $MoPacC^{80}$, all the other three forms could bind to the *cis*-element 5'-GCCAAG-3'. Bioinformatics analysis revealed that thousands of genes in the rice blast fungus genome have the *cis*-element in their promoter. Microarray analysis showed that 156 and 190 of the genes were up or down regulated, respectively, in the MoPacC deletion mutant, suggesting that MoPacC is a transcription repressor and a transcription activator. To determine which form is response for the transcription activation and the transcription repression, transcription activation assay was performed. Only $MoPacC^{222}$ was confirmed to exhibit the transcription activation activity. Besides, four transcription factors were demonstrated to function downstream of the MoPacC to control the vegetative hyphal growth, vegetative melanin biosynthesis and conidiation and the biotrophic growth.

PS04-232

Fungal small RNAs act as effectors to suppress host immune responses

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Small RNAs (sRNAs) are a class of short non-coding regulators that mediate gene silencing in a sequence-specific manner by loading into Argonaute protein (AGO) to target complementary genes. In fungi, although RNAi has been applied as a genetic tool to suppress target gene expression, the natural role of endogenous sRNAs remains enigmatic. Studies in the fission yeast and Neurospora crassa revealed functions of sRNAs in genome defense, heterochromatin formation, and gene regulation. However, it has never been shown that sRNAs or RNAi are directly involved in pathogenicity.We have identified several sRNAs of Botrytis cinerea that can potentially target important regulatory genes in plant hosts, including Arabidopsis and tomato. A majority of these predicted targets were down-regulated by Botrytis infection. Transient co-expression of Botrytis sRNAs and host targets with wild type or mutated target sites confirmed that the suppression of the targets was Botrytis sRNA-specific. Transgenic plants expressing Botrytis sRNAs down-regulate these targets and display enhanced susceptibility. We hypothesize that these fungal-derived sRNAs silence host targets by associating with host AGOs. In the Arabidopsis AGO1 immunoprecipitation fraction, we detected a 21 nt Botrytis sRNA that targets two host MAPK genes, which supports our hypothesis that Botrytis sRNAs function through host RISC during infection. Pathogens deliver effector proteins into host cell to hamper host immune responses and achieve pathogenicity. Here, we discovered that some fungal sRNAs function as effectors to silence regulatory genes of host immunity and contribute to fungal pathogenicity.

PS04-233

Functional analysis of Asian soybean rust resistance pathways

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Historically, the capacity to perform high-throughput genetic and molecular analyses of the crop species *Glycine max* (soybean) has been hindered by the lack of genomic information and tools to assess gene function. The development of a virus-induced gene silencing (VIGS) system for use in soybean coupled with the completed genome sequence has made it possible to functionally analyze genes involved in a wide array of physiological responses, including defense. We are interested in the signaling pathways that enable resistant soybean lines to defend themselves against the highly-virulent obligate biotrophic fungus *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust. To date, five genes, including *Rpp2*, that confer resistance to specific isolates of *P. pachyrhizi* have been identified. *Rpp2*-mediated resistance limits the growth of the pathogen and is characterized by the formation of reddish-brown lesions on the leaf surface and limited uredinia production. Using VIGS we screened 140 candidate genes to identify those that play a role in *Rpp2*-mediated resistance toward *P. pachyrhizi*. Candidate genes included putative orthologs to known defense-signaling genes, transcription factors, and genes previously found to be upregulated during the *Rpp2* resistance response. We identified 11 genes that compromised *Rpp2*-mediated resistance when silenced, including *GmEDS1*, *GmNPR1*, *GmPAD4*, *GmPAL1*, five predicted transcription factors, an O-methyl transferase, and a cytochrome P450 monooxygenase. Together, our results provide new insight into the signaling and biochemical pathways required for resistance against *P. pachyrhizi*. We are currently assessing the function of these 11 genes in soybean accessions containing the other known *Rpp* genes.

PS04-234

A complex genetic system underlies the wheat powdery mildew *Pm3 - AvrPm3* interaction

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We are studying the interaction of the wheat *Pm3* resistance alleles with the corresponding *Avr* genes in the powdery mildew (*Blumeria graminis* f.sp. *tritici*) pathogen. While on the host side, a set of 17 functional *Pm3* alleles has been molecularly isolated, none of the corresponding *Avr* genes has yet been cloned. We have constructed two genetic mapping populations for a map-based cloning approach of several *AvrPm3* genes. Both mapping populations share a common parent, isolate 96224, which was sequenced by 454 and a complete physical map consisting of BAC clones is available.Mapping results for several *Avr* genes reveal a highly complex genetic mechanism. The five analyzed *AvrPm3* genes behave genetically different, although there are genetic loci which are common to avirulence of several *AvrPm3* genes. The *AvrPm3-f* gene segregated as a single locus in the first mapping population and was previously localized in a genomic interval of 30 kb. However, none of the *AvrPm3-f* candidate sequences could be functionally validated. This could be explained with the recently obtained mapping results of the second population, showing that two genes are involved in *AvrPm3-f* avirulence. We conclude that the mechanism of avirulence is more complex than expected based on the gene-for-gene hypothesis and that more than one gene can be required for avirulence. Sequencing of the other two parental isolates by Illumina will now allow the development of high throughput SNP-based genetic maps to perform the map-based cloning of several loci and better understand the molecular basis of these genetic observations.

PS04-235

Identification of genes required for Cf-dependent hypersensitive cell death

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Identification of hypersensitive cell death (HCD) regulators is essential to dissect the molecular mechanisms underlying plant disease resistance. In this study, combined proteomics and RNA interfering analyses were employed to identify genes required for the HCD conferred by the tomato resistance gene *Cf-4* and the *Cladosporium fulvum* avirulence gene *Avr4*. Forty nine proteins differentially expressed in the tomato seedlings mounting and those not mounting the Cf-4/Avr4-dependent HCD were identified through proteomics analyses. Among them were a variety of defence-related proteins including a cysteine protease Pip1, an operative target of another *C. fulvum* effector Avr2. Additionally, glutathione-mediated antioxidation is a major response to the Cf-4/Avr4-dependent HCD. Functional analysis through *Tobacco rattle virus*-induced gene silencing and transient RNAi assays of the chosen sixteen differentially expressed proteins revealed that seven genes, which encode Pip1 homolog NbPip1, a SIPK type MAP kinase Nbf4, an asparagine synthetase NbAsn, a trypsin inhibitor LeMir-like protein NbMir, a small GTP-binding protein, a late embryogenesis-like protein and an ASR4-like protein, were required for the Cf-4/Avr4-dependent HCD. Furthermore, the former four genes were essential for the Cf-9/Avr9-dependent HCD; *NbPip1*, *NbAsn* and *NbMir* but not *Nbf4* affected a nonadaptive bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*-induced HCD as well in *Nicotiana benthamiana*. These data demonstrate that Pip1 and LeMir may play a general role in HCD and plant immunity, and application of combined proteomics and RNA interfering analyses is an efficient strategy to identify genes required for HCD, disease resistance and probably other biological processes in plants.

PS04-236

Innate immunity elicitors from ascomycete *Leptosphaeria maculans* induce resistance in oilseed rape

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Plant innate immunity system can be stimulated by various elicitors, which could be either integral constituents of pathogen body (PAMPs) or secreted during pathogenesis process, e.g. toxins, peptides, effector molecules, etc. A number of such compounds are referred to general elicitors activating host defence responses effective against the vast majority of invading microbes. Our work was aimed at searching for elicitors produced by ascomycete *Leptosphaeria maculans* as well as for possible PAMPs derived from cell walls of this pathogen, inducing resistance in oilseed rape. *L. maculans* was cultivated in vitro in a liquid medium. Both cultivation medium and mycelium was used as a source of elicitors. Application of the medium on cotyledons elevated transcriptional level of genes associated with the biosynthesis of hormones implicated in defence signalling (*ICS1, ACS2, AOS*) that were previously found expressed in *L. maculans* infected plants, as well as induced resistance to *L. maculans* on cotyledons. Following fractionation of the medium using IEF indicates the highest abundance of proteinaceous elicitors in range pH 4.2-4.4. Possible PAMPs were separated from *L. maculans* mycelium using homogenization, ion-exchange chromatography and characterized by enzymatic digestion. Mycelial elicitor both increased expression of defence genes (*PR1, ICS1*) and induced resistance to *L. maculans* on cotyledons of oilseed rape. Elicitor cleavage by α/β-glucosidases resulted in a significant decrease in eliciting capacity, which indicates that the elicitors are prevalently of the polysaccharide nature.

PS04-237

Characterization of an ammonium transporter PiAMT1 from the root endophytic symbiont *Piriformospora indica*

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Nitrogen plays an important role during plant colonization in both mutualistic and phytopathogenic fungi. Whereas it is believed that ammonium is the potential nitrogen source delivered to the host by the mycorrhizal fungi during symbiosis, ammonium limitation has been proposed to act as a key signal to trigger the in planta expression of virulence genes in pathogenic fungi. The root endophyte *Piriformospora indica* displays a biphasic lifestyle during colonization of barley roots with an early biotrophic phase followed by a cell death associated phase. Whole genome analyses of *P. indica* revealed the presence of two different ammonium transporters (PiAMT1 and PiAMT2). No sequences related to nitrate transporters (NRT) could be found in the draft genome. PiAMT1 proved to be highly up-regulated during colonization of barley roots in planta under ammonium limitation condition. We propose that in response to nitrogen starvation PiAMT1 senses the environment and induce signaling in the symbiotic interaction between *P. indica* and its plant hosts. In order to prove this hypothesis we have started a study on clarification of the ammonium transporter PiAMT1 in *P. indica*.

PS04-238

ACRTS1* and *ACRTS2* genes required for biosynthesis of host-selective ACR-toxin in the rough lemon pathotype of *Alternaria alternata

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Host-selective ACR-toxin is produced by the rough lemon pathtype of *Alternaria alternata* and the HST-producing pathogen causes Alternaria leaf spot disease to common root stocks of rough lemon and a hybrid of rough lemon and acid mandarin, rangpur lime. The chemical structure of the major form of ACR-toxin (ACR-toxin I) is a 19 carbon polyalcohol with a α-dihydropyrone ring, a structural feature of typical polyketides. We identified ACR-toxin biosynthesis gene cluster (*ACRT*) carrying in a single small chromosome with the size of 1.5 Mb in the genome of the rough lemon pathotype of *A. alternata*. Using mass sequencing, we isolated two genes; one named *ACRTS1* encoding a putative hydroxylase and other named *ACRTS2* encoding a putative polyketide synthase. Functional role of both *ACRTS1* and *ACRTS2* in ACR-toxin production was examined by target gene disruptions and RNA silencing. Although both genes have multiple paralogs, one or two copy disruption of these genes reduced transcription and ACR-toxin production, and RNA silencing-oriented knock-down mutants did not show any transcript of these genes, ACR-toxin production and pathogenicity to rough lemon. These results indicated that *ACRTS1* and *ACRTS2* are the essential genes for ACR-toxin biosynthesis in the rough lemon pathotype of *A. alternata* and is required for full virulence of this fungus.

PS04-239

Genetic characterization of a novel inhibitor gene in *Capsicum annuum* that represses host specific disease resistance for *Phytophthora capsici*

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A novel disease resistance inhibitor gene (*I*) found in the *Capsicum annuum* accession NMCA10399 inhibits resistance to *Phytophthora capsici*. When *P. capsici* resistant material was hybridized with NMCA10399, the resultant F_1 population was 100% susceptible to *P. capsici* for both root rot and foliar blight disease syndromes. The F_2 population displayed a 3:13 (resistant:susceptible) ratio. The backcross population using the resistant parent displayed an 1:1 ratio, and a backcross population with NMCA10399 as the backcross parent displayed 100% susceptibility. These results demonstrate the presence of a single dominant inhibitor gene affecting the expression of *P. capsici* resistance in *C. annuum*. Moreover, NMCA10399 was tested for its effect on non-host resistance against different *Phytophthora* species. When NMCA10399 was challenged against seven *Phytophthora* species, the *I* gene was only functional against *P. capsici*. These results indicate that *I* is interfering with the expression of specific resistance, but not the expression of nonhost resistance. Further study of *I* should reveal the molecular characteristics of this phenomenon that inhibits resistance against *P. capsici*. The study of NMCA10399 at a molecular level will provide new insights into the *C. annuum-P. capsici* pathosystem and provide information to explain the resistance (defense) mechanism, which could lead to a greater understanding of host resistance.

PS04-240

The wound induced AP2/ERF domain transcription factor WRERF50 confers resistance to necrotrophic fungi, independent of salicylate, ethylene and jasmonate signaling pathways in Arabidopsis

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Necrotrophic pathogens are an agriculturally important group of destructive pathogens. One of them is *Sclerotinia sclerotiorum*, which attacks more than 400 plant species and is one of the most important diseases in oil crops in the world, *S. sclerotiorum* is also the major diseases of oilseed rape in China. But little is known about molecular mechanisms of host resistance to *S. sclerotiorum*, which thus limited development of resistance improvement strategy. We identified WRERF50 gene from cDNA microarray of *B. napus* inoculated with *S. sclerotiorum*. To clarify regulation of WRERF50 gene expression in response to *S. sclerotiorum* infection, wild-type Arabidopsis were treated with wounding and plant hormone ethephon, MeJA and SA and then WRERF50 expression of different treatment and different time points were analyzed by quantitative RT-PCR; in addition, we also detected WRERF50 expression in three mutant backgrounds (*npr1-1*, *coi1-1* and *ein2-1*). The results suggested that expression of WRERF50 was induced by wounding, independent of ET, JA, and SA signaling pathway. Consistently, Over-expression of WRERF50 activates expression of several PR genes *PDF1.2*, *ChiB* and *PR-2*, increased plant resistance against necrotrophic fungi *Botrys cinerea* and *Sclerotinia sclerotiorum* while WRERF50-silencing plants down-regulated expression of several PR genes and decreased resistance to both pathogens.

PS04-241

Proteomics and phosphoproteomics of *Phytophthora infestans* life stages

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Phytophthora infestans is a devastating plant pathogen that can

cause immense damage to a potato field in a week. The cost of *P. infestans* control and damages is estimated to 900 million Euro per year in the EU. An improved understanding of the mechanism of infection of *P. infestans* on a molecular level would be useful for developing novel methods of pathogen control measures. We have used proteomics to study *P. infestans* life stages in order to identify unique proteins and protein phosphorylation events. Previously, a microarray approach has been used to study mRNA levels during various life stages. However, since the correlation between mRNA and protein levels is rather low, with levels of mRNA explaining approximately 40% of the variation in protein levels, it is of interest to study protein levels directly. To our knowledge this is the first large scale proteomics and phosphoproteomics study of *P. infestans*. Using qualitative and quantitative proteomics, we have identified more than 4000 *P. infestans* phosphopeptides and 2000 phosphorylation sites. Among the identified phosphoproteins are a number of proteins involved in infection such as members of the CRN-family of effector proteins, not previously described as phosphoproteins. From the phosphosites, we have identified phosphorylation motifs, some of which are previously undescribed. In addition, we have acquired quantitative data for 1500 proteins and more than 4000 phosphopeptides in the different life stages. Among these are a number of proteins specific for life stages of particular interest for the infection process.

PS04-242

Effect of Methyl jasmonate on the suppression of gray mould disease and on *PAL* defense gene expression in *Botrytis cinerea* infected grapevine berries

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Botrytis cinerea, a necrotrophic pathogen, causes gray mould in grapevine (*Vitis vinifera*). Methyl jasmonate (MeJA) occurs naturally in host plant tissues and has signalling role in eliciting induced systemic resistance (ISR) against disease. This study investigates the effect of exogenous MeJA, on the suppression of postharvest gray mould in green grape cultivars Chardonnay and Vidal and in red grape cultivars Merlot and Cabernet Sauvignon. The grape bunches (15 grapes/bunch and three replicate treatments) were spray-treated with 1mM of MeJA, air dried for 3 hours. Three days after the MeJA treatment, each of the grape berry in the bunch was wounded with a needle and inoculated with 1 x 10^{4} spores of *B. cinerea* B05.10 and incubated in the dark at 20 ºC and 85% RH. Control treatment did not receive MeJA. The lesion diameter was recorded at 7 and 14 days after inoculation. The elicitor, MeJA induced defense response by significantly suppressing the *Botrytis* gray mould disease in all the grape cultivars tested. Defense response, expressed as *PAL* gene, in grapevine berries towards *B. cinerea*, was studied. Maximum levels of induction of *PAL* gene was observed at 48 hpi in *B. cinerea* infected, MeJA treated, or MeJA treated and *B. cinerea* infected grapevine berries. A significantly lower level of *PAL* gene expressed in MeJA treated and *B. cinerea* infected grapevine berries, as compared to *B. cinerea* only infected berries. Postharvest treatment with methyl jasmonate may be incorporated as a potential tool in the grape postharvest disease management strategies.

PS04-243

Loss of function of ethylene receptor ETR1 in *Arabidopsis* reduces *Fusarium oxysporum* infection

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Fusarium wilt disease, caused by *Fusarium oxysporum*, is a common disease of a wide range of economically important crops that is difficult to control, resulting in severe yield losses. The responses of *Arabidopsis thaliana* mutant plants impaired in known pathogen response pathways were used to explore the components in defence against *F. oxysporum*. Pathogenicity experiments of the mutant lines with *F. oxysporum* revealed enhanced resistance in *etr1-1* [ethylene (ET) receptor mutant] plants, but not in salicylic acid-, jasmonic acid or other ET-deficient mutants, indicating a crucial role of ETR1 in defence against this pathogen. Quantification of the pathogen in plant tissues by qPCR revealed that the decrease in symptom severity shown in *etr1-1* plants was associated with significant reduction in the growth of the pathogen in the vascular system of the plants, suggesting that impaired perception of ET via ETR1 results in increased disease resistance. Furthermore, gene expression analysis of several defence genes showed elevated expression levels of the PR1, PR2 and PR5 transcripts in *etr1-1* plants after *F. oxysporum* inoculation. The latter indicates that the induced defence responses of *etr1-1* plants are dependent on a set of defence genes activated on pathogen attack.

PS04-244

Controlling Perilla rust using plant-derived essential oils

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Perilla is used extensively as a leafy vegetable or a cuisine oil due to the distinctive aroma and pungency. As the most damaging disease lowering the quality of perilla leaves, perilla rust has been controlled with limited numbers of agrochemicals due to the property of leafy vegetable. Therefore, new methods of controlling the disease environmentally friendly are required. Essential oils are natural compounds derived from plants, which contain volatile aroma with antifungal activities. A newly developed method was applied for higher extraction of the essential oils from dried leaves and from seeds, and the extracted essential oils had the controlling activity of fungal diseases. Further investigation is undergone for identifying the mechanism of controlling the fungal diseases, including the perilla rust.

PS04-245

Progress on the cloning of *ATR2* from *Hyaloperonospora arabidopsidis*

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Hyaloperonospora arabidopsidis (Hpa) is a natural biotrophic pathogen of *Arabidopsis thaliana* and the interaction phenotype with its host is determined by the pathogen originated *ATR* (*Arabidopsis thaliana* recognized) and the corresponding host *RPP* (recognition of *Peronospora parasitica*) genes. Arabidopsis Col-0 carries *RPP2A* and *RPP2B* (Sinapidou et al., 2004, Plant J. 38: 898-909), which enables recognition of the avirulence determinant designated ATR2Cala2 from Hpa-Cala2. We screened an F2 population generated from the cross between Hpa-Cala2 and Hpa-Noks1, which was previously used to clone ATR5Emoy2 (Bailey et al, 2011, MPMI 24: 827-838) and identified a genetic interval

for semi-dominant ATR2Cala2. A physical map of ATR2Cala2 has been established using the publicly available genomic and BAC sequences. We then screened 192 F2 isolates and established an interval for the ATR2Cala2 locus of 188kb. Illumina paired-end sequencing data for Hpa-Cala2 and Hpa-Noks1 were generated and used to identify polymorphic markers enabling us to narrow the interval down to 40kb. None of the genes within the interval possess an RXLR motif, but there are putative secreted proteins, which show gene duplication. Currently, the candidates are being tested and the latest data will be presented.

PS04-246

Rpiblb2-mediated late blight resistance requires SGT1 and salicylic acid-mediated signaling, but not RAR1 or HSP90, in *Nicotiana benthamiana*

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Potato (*Solanum bulbocastanum*) *Rpiblb2* encodes a protein with a putative CC-NBS-LRR (coiled-coil-nucleotide binding site and leucine-rich repeat) motif that confers *Phytophthora* late blight disease resistance. We examined the components required for *Rpiblb2*-mediated resistance to *P. infestans* in *Nicotiana benthamiana*. *Tobacco rattle virus* (TRV)-induced gene silencing (VIGS) was used to repress candidate genes in *N. benthamiana* and to assay against *P. infestans* infections. NbSGT1 was required for disease resistance to *P. infestans* and hypersensitive responses (HRs) triggered by coexpression of AVRblb2 and Rpiblb2 in *N. benthamiana*. RAR1 and HSP90 did not affect disease resistance or HRs in *Rpiblb2*-transgenic plants. To elucidate the role of salicylic acid (SA) in Rpiblb2-mediated resistance, we analyzed *NahG*-transgenic plant responses following *P. infestans* infection. The increased susceptibility of transgenic Rpiblb2 plants on the *NahG* background correlated with reduced SA and SA glucoside levels, but did not correlate with HR cell death induction. Furthermore, Rpiblb2-mediated HR cell death was associated with H_2O_2, but not SA, accumulation. SA is required for basal defense and for Rpiblb2-mediated resistance against *P. infestans*. These findings provide insight into the roles of SGT1 and SA in Rpiblb2-mediated disease resistance against *P. infestans*.

PS04-247

The role of *VdSteA* G protein coupled pheromone receptor in virulence and biology of the vascular wilt pathogen *Verticillium dahliae*

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V. dahliae is a soil-borne fungus causing wilt diseases in several hosts. The particular biology of this fungus complicates its treatment through conventional methods. Thus, the study of genes implicated in interactions of the fungus with its hosts is necessary to unravel the pathogenicity or virulence mechanisms and to discover putative novel methods to control the disease. G Protein-Coupled Receptors (GPCRs) represent the largest family of transmembrane receptors consisting of seven transmembrane domains. GPCRs are critical factors in regulating morphogenesis, defense, mating, infection and virulence in various organisms. Protein sequences of characterized GPCRs of the well studied fungi *Aspergillus nidulans* and *Magnaporthe grisea* were used for alignment comparison with the genome of *V. dahliae* in order to detect potential GPCRs. After performing phylogenetic analysis, the sequences of *V. dahliae* that showed high homology to the GPCRs of *A. nidulans* and *M. grisea* were selected in order to sort out the receptors by their molecular relativity. Seven different groups of GPCRs emerged from the phylogenetic analysis, varying in sensing different environmental signals. *Agrobacterium* mediated disruption of a pheromone GPCR (named as *VdSteA*) in two wild type races, 70V and 25V of *V. dahliae* was performed in order to study the role of this receptor in virulence and morphology. 70V and 25V *DVdSteA* mutants displayed reduction in virulence in eggplants and tomato plants and 70V *DVdSteA* mutants exhibited increased microsclerotia formation and conidiation compared to their corresponding wild types. Both *DVdSteA* mutants exhibited higher conidial germination rates compared to the wild types.

PS04-248

The Necrosis and Ethylene inducing Protein (VdNEP) gene is implicated in symptom induction by the vascular wilt fungus *Verticillium dahliae*

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VdNEP belongs to a NLP (NEP1 like proteins) family that contains nine identified genes and has been shown to induce leaf necrosis and defense responses on several hosts. In the present study, *VdNEP* was investigated for its involvement in symptom induction and virulence of *V. dahliae*. To this end, the *VdNEP* gene was overexpressed in multiple *V. dahliae* strains using two constitutive fungal promoters (*Aspergillus nidulans*-trpC and *Magnaporthe oryzae*-RP). Increased necrosis symptoms on cotton plants were observed when *VdNEP* was overexpressed in transformants of the *V. dahliae* cotton defoliating and non-defoliating pathotypes. Similarly, inoculation of tomato plants with the same cotton defoliating transformant overexpressing *VdNEP* caused stunting and increased necrosis symptoms. In contrast, the wild type defoliating strain, which is less virulent on tomato than cotton, caused weak chlorosis and wilting symptoms and hyperauxiny, as tomato plants grew taller compared to uninoculated control plants. Moreover, transient expression of *VdNEP* in tomato plants via a TRV (*Tobacco rattle virus*)-expression vector of *VdNEP* caused typical necrosis symptoms. Results of the present study suggest the implication of *VdNEP* in symptom induction by *V. dahliae*.

PS05-249

Interaction of biological control agent *Serratia plymuthica* A30 with blackleg causing biovar 3 *Dickeya* spp. *in vitro* and *in planta*

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In Europe pectinolytic bacteria belonging to *Dickeya* spp. cause increasing losses in (seed) potato production. This is related to presence of a new, unclassified genetic clade of biovar 3 *Dickeya* spp. provisionally named *D. solani*. Effective strategies to control *Dickeya* spp. have not been developed yet. We have characterized a biological control agent *Serratia plymuthica* strain A30, an endophyte isolated from rotten potato tuber tissue and active against *D. solani*. This antagonism requires direct contact between the control agent and the pathogen and is most likely based on antibiosis. In a potato slice assay, strain A30 eliminated the pathogen and prevented potato tissue maceration by *D. solani* when inoculated in densities at least 100 times higher than the pathogen. To study the interaction between *S. plymuthica* A30 and *D. solani*

in planta, fluorescent protein tagged strains (marked with GFP and DsRed) were exploited. In repeated greenhouse experiments, a tuber treatment with strain A30 protected potato plants against *D. solani* effectively, resulting in a decrease in the incidence of stem infection of, on average, 97%. Using confocal laser scanning microscopy, the antagonist could be traced in vascular and parenchymatic tissue of tubers, roots and stems at least till 28 days after planting. Results indicated that *S. plymuthica* A30 outcompeted *D. solani in planta*. We used random transposon mutagenesis and genome analysis to characterize potential genes of *S. plymuthica* A30 involved in biocontrol.

PS05-250

Consortia of environmentally friendly microbial for control blast, bacterial leaf blight, and sheath blight diseases on rice plants

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The use of bacteria as biocontrol agents environmentally friendly need to be explored. This study was aimed to (a) study the inhibitory ability of eight isolates of biocontrol bacteria against plant pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) as the cause of bacterial leaf blight disease (BLB), *Rhizoctonia solani* as the cause of sheath blight disease, and *Pyricularia oryzae* as the cause of blast disease, (b) determine the effectiveness of the consortia of bacteria to control the diseases, and (c) *in vivo* application of biocontrol agents formulative composed by talcum, bentonite, vegetable oil, and suspension as carrier agent on cultivar IR 64 rice plants in greenhouse. Isolates which used as biological control are *Pseudomonas aeruginosa* C32a and C32b, *P. fluorescens* Pf, *Serratia marcescens* E31, *Bacillus* sp. I.5, *Bacillus cereus* I.21 and II.14, and *B. firmus* E65. The research method consists of testing hypersensitivity, test of antagonistic to Xoo, and *in vivo* application of biological control isolates in the greenhouse. Antagonist test of C32a, C32b, and I.5 showed inhibitory activity against Xoo. Application of C32a isolate could suppress the long of wound BLB better than chemical agent. Among of eight treatments, the results of compatibility test found the best formula to inhibit *R. solani* was A2 consisted E65, and A8 formula using bentonite as carrier consisted of a mixture of E65, E31 C32b, and II.14. A2 treatment used E65 and A6 treatment used E65, II.14, and C32b showed the best inhibition against *P. oryzae* i.e. 73-85% and 66-83%, respectively. Key words: rice, biocontrol, blast

PS05-251

***Pseudomonas fluorescens* SBW25 secretes a biosurfactant that facilitates sliding motility and plant growth promotion**

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Pseudomonas fluorescens bacteria are common soil inhabitants that favour colonisation of plants, especially the root environment (rhizosphere). *P. fluorescens* strain SBW25 has been extensively studied to understand the genetic basis of its ecological success in the rhizosphere. The flagellum master regulator, FleQ, is important for negatively regulating *wss* genes (encoding cellulose extracellular polysaccharide (EPS)) and positively regulating flagellar genes. This indicates that FleQ is probably important for transitional switching of the bacterial lifecycle from the motile planktonic form (in the soil) to the non-motile EPS-producing biofilm form on and within plant tissues. It was also discovered that FleQ plays a role in bacterial surface-spreading motility: mutation of *fleQ* in SBW25 (SBW25del*fleQ*) revealed a flagellum-independent surface-spreading motility phenotype. Mutagenesis of SBW25del*fleQ* identified several non-motile mutants. PCR analysis identified the mutations to two non-ribosomal synthetase genes known to be involved in production of the biosurfactant viscosin. Complementation of these mutants with *fleQ* restored surface motility despite a lack of viscosin production. This indicates that SBW25 can move over surfaces by flagellum-dependent swarming and viscosin-dependent sliding motility. We also investigated whether viscosin might improve plant growth in the presence of oomycete and fungal pathogens. Plant growth promotion assays using SBW25del*fleQ* viscosin mutants showed that viscosin is the key bacterial product responsible for suppression of oomycete and fungal detrimental effects on plant seedling emergence and development. Taken together, our data have uncovered the major factor that is responsible for *P. fluorescens* SBW25 suppression of plant root pathogens.

PS05-252

***In silico* analysis of transcriptional regulatory elements related with disease resistance**

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Plant utilizes diverse and sophisticated signaling cascades for recognizing and responding to a wide range of biotic and abiotic stresses. Induced systemic resistance (ISR) is a phenomenon whereby resistance to infectious disease is systemically induced by localized infection or treatment with microbial components such as plant growth promoting fungi (PGPF). Multiple defense signals are induced by the PGPF, *Penicillium simplicissimum* GP17-2 against *Pseudomonas syringae* pv. *tomato* DC3000 (Pst). Culture filtrate (CF) of PGPF-treated plants infected with the pathogen exhibited elevated expression of thousands of defense- related genes which are identified by microarray analysis. Different phytohormone activities are involved in the transcriptional regulation of this signal transduction of defense responsive genes. The present study was aimed to identify the PGPF- mediated ISR responsive elements in the promoter of stress inducible genes to dissect integrated transcriptional network where multiple hormones are supposed to be committed. Prediction of putative transcriptional regulatory elements was made with the help of bioinformatics study from the promoters identified from public (responsive to SA) and our own microarray data (responsive to H_2O_2 and CF), and synthetic plant promoters were prepared by using the predicted putative cis-regulatory elements to diagnose the regulatory responses of the elements in transcriptional network. Cross-detection of the same elements suggests their possible crosstalk. Precise analysis of cis-acting elements and their transcription factors can give an accurate understanding of regulatory systems in stress-responsive gene expression.

PS05-253

Genes expressed in tissue-cultured seedlings of mountain laurel (*Kalmia latifola* L.) with colonizing *Streptomyces padanus* AOK30

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Endophytic actinomycete, *Streptomyces padanus* AOK30 is capable

of protecting mountain laurel against infection by *Pestarotiopsis sydowiana*, a causal agent of Pestalotia disease, when applied to the seedling of the plant. In this study, suppression subtractive hybridization (SSH) was used to identify genes differentially expressed in seedlings of mountain laurel after application of *S. padanus* AOK30. Subsequent dot hybridization with independent RNA from *S. padanus*-colonized and control plants identified non-redundant 181 cDNAs involving 72 and 109 clones, which were up- and down-regulated upon inoculation with the bacteria, respectively. Comparison of the sequences with databases revealed that a number of transcripts encoding proteins or enzymes that function directly in defense or stress response and regulatory proteins were regulated differentially in the seedlings with colonizing *S. padanus* AOK30. Semi-quantitative RT-PCR analysis for the selected genes demonstrated that inoculation of mountain laurel seedlings with *S. padanus* AOK30 increased expression of defense-related genes as well as distinct classes of glutathione *S*-transferase, although endochitinase were exclusively suppressed. These results clearly indicate that the *S. padanus*-colonizing seedlings likely initiate or prime plant defense responses towards pathogen infection. Differential expression of the selected genes was also observed in *S. padanus*-colonized seedlings, compared to those solely challenged with a fungal pathogen, *P. sydowiana*. This approach will assist in efforts not only to understand the molecular basis of the enhanced tolerance and/or enhanced disease resistance of mountain laurel, but to define a core set of genes during colonization or association with *S. padanus* AOK30.

PS05-254

Induced resistance and antibiosis a dual mode of action of *Pseudozyma aphidis* against diverse phytopathogens

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Plant pathogens challenge our efforts to maximize crop production due to their ability to rapidly develop resistance to pesticides. This can result in immense yield losses on an annual basis. One of the main research goals of this century involves the development of new tools to control pathogens. Fungal biocontrol agents have become an important alternative to the use of chemicals due to environmental concerns. We recently isolated the epiphytic yeast-like *Pseudozyma aphidis* from strawberry leaves. Our data suggest that this *P. aphidis* isolate secretes extracellular metabolites which inhibit several plant pathogens *in vitro*. In addition, application of the *P. aphidis* spores on plants in the greenhouse significantly reduced *Botrytis cinerea*, *Clavibacter michiganensis* or powdery mildew infection. We also demonstrated that *P. aphidis* can sensitize the plant's defense machinery by induction of *PR1* and *PDF1.2* gene expression locally and systemically in Arabidopsis plants. We further found that *P. aphidis* could reduce *B. cinerea* infection in Arabidopsis mutants impaired in JA or SA signaling, *jar-1-1* and *NahG* and *npr1-1* locally and systemically. This suggests that above the direct inhibition *P. aphidis* inhibit *B. cinerea* infection also by induced resistance in SA-, JA- and NPR1-independent manner. Moreover we found it cannot reconstitute *PR1* and partially reconstitute *PDF1.2* expression in the mutants systemically, suggesting the induced resistance ability of *P. aphidis* is not directed solely through *PR1* and *PDF1.2* but probably also through other different pathogenesis resistance genes and/or pathways as well.

PS05-255

The biocontrol strain *Pseudomonas fluorescens* F113 is toxic towards soil amoebae

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Pseudomonas fluorescens F113 is able to protect crops such as beetroot and tomato from phytopathogenic fungi. While interacting with the plant host, biocontrol strains are exposed to predation by bacteriophagous invertebrates such as protozoa. Therefore, rhizospheric bacteria may have evolved molecular mechanisms to face this ecological pressure. We tested the ability of the protozoan *Acanthamoeba polyphaga* to graze on *Pseudomonas fluorescens* F113. *A. polyphaga* was unable to feed and multiply on *P. fluorescens* F113 wild-type. However *gacA* or *gacS* mutants (*P. fluorescens* F113 derivatives lacking secondary metabolites) supported amoebal growth but did so to a lesser extent than a harmless *Escherichia coli* strain. At the cellular level, *A. polyphaga* in co-culture with F113 wild-type emitted long filopodia prior to cell death, and interaction with the *gacA* mutant showed similar effects of cytoskeletal changes. Our results indicate that *P. fluorescens* F113 possesses Gac-dependent and Gac-independent mechanisms of toxicity towards *A. polyphaga*.

PS05-256

Suppression of Fusarium wilt disease by an organic hydroponics system

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Organic hydroponics, capable of mineralising organic compounds to inorganic nutrients by constructed microbial community in the water, shows a potential to suppress root diseases. To assess suppressive effect against root diseases, Firstly, *in vitro* examination of suppressive effect of the hydroponic solution from organic hydroponics was conducted using a fungal pathogen *Fusarium oxysporum*, causing Fusarium wilt of lettuce. This result demonstrated that the density of *F. oxysporum* was dramatically increased in sterilized hydroponic solutions by filtration or autoclaving, respectively. On the other hand, the growth of *F. oxysporum* was suppressed in an untreated hydroponic solution containing living microbial community. Secondly, we conducted an inoculation test on lettuce seedlings with *F. oxysporum*. A conventional hydroponic system, which is required to use only inorganic nutrients, showed severe disease symptoms on cultivated seedlings. In contrast, organic hydroponics showed no disease symptoms although *F. oxysporum* was detected from surface of the plant roots and the hydroponic solution. However, suppressive effect of the Fusarium wilt was not observed when the fungal pathogen was inoculated within 3 days after transplanting while fully developed after that period. Finally, we conducted DGGE analysis to reveal the microbial composition of rhizosphere biofilms from organic hydroponics. In this result, transitions of microbial composition were observed before and after plant cultivation. Altogether, considering *in vitro* and *in planta* experiments, suppressive effect on a Fusarium wilt disease was characterised into suppression of pathogen growth and infection to plant roots.

PS05-257

Analysis of microbial community in organic hydroponics solution

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Organic hydroponics is an epoch-making culture method of using organic matters as fertilizer, which is degraded by microbial ecosystem constructed in the hydroponic solution. Multiple parallel mineralization, which is continuous reactions of ammonification

and nitrification in water, enables cultivation of vegetables by adding organic fertilizer directly to the hydroponic solution. This hydroponics system has suppression effect of root disease such as *Fusarium* wilt and bacterial wilt disease, however characteristic biofilm developed on the surface of roots has not been analyzed sufficiently. In this study, we examined succession of microbial ecosystem in the biofilm, dominant microbial strain at each stage and effect of adding organic fertilizer on microbial ecosystem. At first multiple parallel mineralization was conducted to construct suitable microbial ecosystem by using two kinds of organic fertilizer and then butterhead lettuce was cultivated using this solution. Biofilms were collected from the wall of cultivation tank and plant roots at each stage and they were analyzed by PCR-DGGE method. As a result, microbial composition of biofilm was changed greatly between collecting points. At the stage of microbial ecosystem construction before cultivation, similar microbial composition, such as *Bacillus* sp. and *Comamonas* sp., was confirmed regardless of using different organic fertilizers. At cultivation stage, difference of microbial ecosystem between organic fertilizers was expanded. These results suggest that plant roots have an effect on selection of microbes to degrade each organic fertilizer effectively.

PS05-258

Successful organic hydroponics by construction of microbial ecosystem in the hydroponic solution and the suppressive effect of bacterial wilt disease

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Hydroponics is an excellent technique for the cultivation of vegetable crops and other plants, but organic fertilisers cannot be used in conventional hydroponic systems, which generally use only inorganic fertilisers, because organic compounds in the hydroponic solutions generally have phytotoxic effects that lead to poor plant growth. Few microorganisms are present in hydroponic solutions to mineralize the organic compounds into inorganic nutrients. However, from the viewpoint of resource recycling, it is important to develop methods capable of using organic fertiliser sources in hydroponics. We developed a novel and practical hydroponic culture method that uses microorganisms to degrade organic fertiliser in the hydroponic solution. Soil microorganisms were cultured by regulating the amounts of organic fertiliser and inoculum, with moderate aeration. The microorganisms mineralised organic nitrogen via ammonification and nitrification into nitrate in water. The culture solution containing the microorganisms was usable as a hydroponic solution, and organic fertiliser could be directly added to it during vegetable cultivation. Vegetables grew well in our organic hydroponic system. Inoculation of *Ralstonia solanacearum*, a phytopathogenic bacteria of bacterial wilt disease, in organic hydroponics resulted in no disease symptoms on tomato seedlings, in contrast to inorganic conventional hydroponics, in which many seedlings became wilted and died. *R. solanacearum* couldn't be detected from both the hydroponic solution and tomato seedlings in organic hydroponics. These results suggest that organic hydroponics system has suppressive effect to bacterial wilt disease.

PS05-259

Transmission of mycoviruses by attenuating programmed cell death in *Rosellinia necatrix*

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Rosellinia necatrix Prillieux cause severe root rot diseases in fruit trees. We have developed a disease protection system called "Virocontrol", in which we use mycoviruses to reduce the virulence of fungal pathogens. The fungal incompatibility system prevents mycoviruses from spreading to fungal strains with different genetic background; therefore, in order to successfully introduce mycoviruses into a given fungal strain, we try to inhibit or attenuate incompatible reaction. The fungal incompatibility reaction is considered to be a type of programmed cell death though its molecular machinery remains to determine. We added various kinds of chemical inhibitors into the culture agar media during hyphal pairing and tested whether mycoviruses were transmitted to the recipient fungal isolates that were mycovirus-free and hygromycin B resistance characters. We treated 87 kinds of chemical inhibitors including cell wall synthesis, protein degradation, phosphorylation, calcium signaling, reactive oxygen species generation, and so on. We found that zinc chloride treatment transmitted several kinds of mycoviruses including *Rosellinia necatrix* megabirnavirus 1 (RnMBV1), one of the potential virocontrol agent. The mycovirus transmission effect was observed not only the treatment with zinc chloride but also with zinc vitriol suggesting that the zinc element was active substance. Microscopic observation revealed that zinc chloride treatment increased hyphal fusion on the incompatible pairing and transmitted cytoplasmic GFP proteins to the opposite fungal isolate.

PS05-260

Multiple host adhesion factors of extracellular matrix (ECM) in *Magnaporthe oryzae*-potential target for disease control-

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The germlings of *Magnaporthe oryzae* are tightly attached on the host surface producing the extracellular matrix (ECM) from germ tubes and appressoria. Spore germlings were treated with various lectins and inhibitors revealed that the glycoprotein(s) consisting of mannose sugar might be important for the adhesion. We also evaluated the effects of hydrophobins, the fungal surface protein, on adhesion and pathogenicity. Gene knockdown and knockout experiments of hydrophobin genes revealed that class I *Mpg1* was involved in adhesion and pathogenicity but class II *Mhp1* was not. Moreover, we found that treatment with natural nutrients such as beef and yeast extract suppressed the appressorium formation and the adhesion that was irrespective of yeast α-factor. By biochemical study, the ECM of *M. oryzae* could be degraded by collageno/gelatinolytic enzymes. We screened gelatinolytic bacteria from rice leaves and soil to establish a novel biological control agent inhibiting germling adhesion on the host plant surface. The selected bacteria were identified as *Acidovorax*, *Sphingomonas*, *Chryseobacterium*, and *Pseudomonas* sp.. Based on the treatment with EDTA, most isolates produced metalloproteinase. The screened bacterial culture showed inhibitory effects on spore adhesion on the plastic cover glass and disease protective effects on rice. However, the selected bacteria could not fix on leaf within 1 week using chloramphenicol resistance marker. We improved bacterial fixation supplemented with 0.3% gelatin and disease protection effect lasted 1 week after bacterial incubation. This study suggests that gelatinolytic bacteria inhibiting germling adhesion may have promise as a biological agent.

PS05-261

Latest generation of biocontrol agents developed by combining agronomic performance and omics techniques

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Beneficial microbes are used to sustain agriculture yields and reduce environment impact. The basic technology has been substantially modified and improved by using a variety of omics techniques, including proteomics and metabolomics. Latest generation of bio-products have a strong scientific base, derived from in detail study of the multiplayer interactions involved (plant-pathogen-biocontrol agent), and are much more effective and reliable. Many novel formulations are now applied as mixtures of living microbes and bioactive molecules, showing activity on the entire plant and being compatible with other bio-products and commonly used pesticides. Knowledge obtained by studying the genome and the mechanism of action of world-wide used antagonistic fungi and bacteria has allowed the development of ready-to-use technology packages to be implemented directly in medium-to-large farms for control of fungi, bacteria, viruses, nematodes and effects abiotic stresses. The new technology, mainly destined to developing countries, has produced a substantial reduction of agrochemical use and has permitted the commercialization of new lines of horticultural products labelled as zero-residue without organic farming. From omics to the field projects have been successfully carried out in Honduras, Costa Rica, Brasil, Perù, Cina, Libya, Venezuela, etc. against diseases of melon, pineapple, strawberry and tomato. Finally, new plant stimulating molecules, including some fungal hydrophobins, have been identified, which are able to activate ISR, promote growth and root development, increase resistance to drought and lack of nutrients, and kill directly several fungal phytopathogens.

PS05-262

Pseudomonas-mediated induced systemic resistance, what is in it for the bacteria

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For many strains of *Pseudomonas* spp. with biological control properties, ISR has been recognized as an important mechanism of disease suppression. *Pseudomonas*-mediated ISR is both plant species specific and bacterial strain specific. In radish *P. fluorescens* strains WCS374 and WCS417 can elicit ISR, whereas *P. putida* strain WCS358 can not, but in Arabidopsis WCS374 can not elicit ISR and both WCS358 and WCS417 can. In Arabidopsis the transcription factor MYB72 is required for effective expression of ISR, as expression of MYB72 is up regulated upon root colonization by ISR eliciting bacteria, and myb72 knock out mutants can no longer express ISR. The root colonizing abilities of the three WCS *Pseudomonas* strains were studied on wild type *A. thaliana* Col-0 and a myb72 knock out mutant in the Col-0 background. Both WCS358 and WCS417 colonized the roots of Col-0 to much higher population densities than WCS374. However, on the myb72 knock out all three strains reached relatively low population densities. Thus it appears that high population densities of ISR eliciting bacterial strains are supported in the rhizosphere of a plant genotype that can express ISR and the bacteria are somehow rewarded. Implications of ISR on recruitment and functioning of the rhizosphere microbiome are discussed.

PS05-263

Insecticidal activity of *Pseudomonas taiwanensis*

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Pseudomonas taiwanensis is a new species isolated from soil and classified recently. In a previous study, the insecticidal activity of *P. taiwanensis* toward *Drosophila melanogaster* larvae was demonstrated and the insecticidal protein gene *tccC* was cloned and heterologously expressed in *Escherichia coli*. The recombinant TccC protein showed high insecticidal activity toward *Drosophila* larvae. In this study, the insecticidal activity of *P. taiwanensis* and the function of TccC were further investigated. *P. taiwanensis* not only showed insecticidal activities against larvae of *Plutella xylostella*, *Spodoptera exigua*, *Spodoptera litura*, *Trichoplusia ni*, and *Drosophila melanogaster* but also induced apoptosis in Sf9 and IPLB-Ld652Y insect cells. In order to assess, an isogenic *tccC* gene knockout mutant of *P. taiwanensis* was generated by replacing the *tccC* gene. As compared with the wild-type strain, the *tccC* gene knockout mutant of *P. taiwanensis* showed lower toxicities toward Sf9 insect cells and *Plutella xylostella* larvae. Inside the *P. taiwanensis* cell, TccC protein might be processed into two fragments, a N-terminal fragment containing a recombinational-hot-spot (Rhs) domain and a C-terminal fragment containing a sodium/glutamate symporter domain and a TraT domain. New studies for evaluating the biological functions of these two fragments derived from TccC are now in progress.

PS05-264

Transcriptomic analysis of systemic resistance induced by a plant growth-promoting fungus *Penicillium simplicissimum* GP17-2

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The plant growth-promoting fungus (PGPF) *Penicillium simplicissimum* GP17-2 induces systemic resistance against bacterial leaf speck caused by *Pseudomonas syringae* pv. *tomato* DC3000. The ISR signaling involves multiple plant hormone-mediated paths, including salicylic acid, jasmonic acid, and ethylene. In this study, we investigated signal transduction for GP17-2-mediated ISR by microarray and promoter analyses. Microarray data of GP17-2 treatment were subjected to comparative analysis with pathogen, plant hormone, hydrogen peroxide and wound responses. Results showed that gene expression at 6 hours post GP17-2 treatment was classified into the same clade with salicylic acid and hydrogen peroxide; in contrast, gene expression at 24 hours post treatment showed only that of abscisic acid. These results suggest crosstalk between ISR induced by GP17-2 and responses of salicylic acid and hydrogen peroxide at earlier stage of ISR, and at later stage, of abscisic acid. Subsequently, we did *in silico* promoter analysis of the identified genes involved in GP17-2-mediated ISR. The promoter prediction method we developed showed much higher success rate and high sensitivity than conventional prediction methods (Yamamoto et al., BMC Plant Boil. 11: 39, 2011). Our prediction provided various putative ISR and also phytohormone-responsive elements. These candidates were applied *in vivo* functional analysis using synthetic promoter and luciferase reporter system. These analyses are expected to provide new knowledge of the transcriptional network of GP17-2-mediated ISR and plant hormone signaling.

PS05-265

Control of rice diseases using an extract of the shrub *Chromolaena odorata* involves induced resistance

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Chromolaena odorata is an invasive weed from the Neotropics, but we have recently found that an aqueous extract of the plant could control important diseases in rice under both controlled and field conditions by application to the seeds before sowing or by spraying on the leaves. Indeed, significant control was obtained of sheath blight (*Rhizoctonia solani*), brown spot (*Bipolaris oryzae*), rice blast (*Pyricularia oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) [Khoa et al. (2011). Phytopathology 101:231-240]. Expression of different defence-related genes was studied in plants inoculated with *R. solani* and included genes encoding PR-proteins [β-1,3-glucanase (PR-2), chitinase (PR-3), thaumatin-like protein (PR-5), peroxidase (PR-9), PR-1b and PBZ1 (both PR-1)] and genes encoding two enzymes involved in the hydrogen peroxide metabolism (superoxide dismutase and catalase). Application of the extract prior to pathogen inoculation resulted in elevated transcript levels of the defence-related genes compared to control plants pre-treated with water. This resulted in decreased fungal growth and reduced formation of infection cushions of *R. solani*. The results indicate that the protection exerted by the extract involves induced resistance since defence responses were enhanced in plants treated with the extract followed by inoculation with the pathogen compared to control plants pre-treated with water. To identify the active compound(s) responsible for the disease-reducing effect, the extract has been fractioned using group separation and analysed by capillary electrophoresis. The sub-fractions are currently being subjected to NMR analyses for structure elucidation of the active compound(s).

PS05-266

Obstacle of "VIROCONTROL": vacuole-mediated programmed cell death during heterogenic incompatibility in *Rosellinia necatrix*.

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The ascomycete fungus *Rosellinia necatrix.* cause severe root rot disease of fruit trees. For sustainable disease protection, we have attempted to develop "Virocontrol", which utilizes hypovirulent mycovirus. For the success of virocontrol, mycoviruses should transfer from hypha to other hypha via anastomosis. However, in these fungi, the heterogenic incompatibility accompanied by active programmed cell death (PCD) prevents mycoviruses from spreading among different fungal strains. In this study, we observed hyphal interaction between compatible and incompatible pairings with light (LM) and transmission electron microscopes (TEM). Mycelial interactions (barrage line) were classified into three types, i.e., broad melanin line, narrow melanin line, and narrow melanin line with highly pigmentation limited one-sided mycelia. LM observation revealed that hyphal anastomosis occurred with high frequency in compatible pairing. In contrast, in incompatible pairing, the anastomosis hardly occurred. We assumed that this fungus released self/nonself-recognition substances. Treatment with activated charcoal suppressed not only barrage line formation in incompatible combination but also hyphal anastomosis in compatible combination. TEM observation of the incompatible hyphae revealed that cell structures degenerated as following order; vacuole, cell membrane, nucleus, endoplasmic reticulum and mitochondria. The degenerated nucleus was characterized by disconnection of nuclear membrane and loss of internal electron density, but heterochromatin condensation did not occur. The heterogenic incompatible PCD was initiated by vacuolar degeneration and followed membrane degeneration of organelles, which were atypical features of apoptosis and autophagy but novel type of PCD.

PS05-267

Development of agricultural material for rice using the ability of rice symbiotic bacteria

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Various kinds of fungus and bacterial endophytes are isolated form plants. Some endophytes have given the useful functions, such as growth promotion, disease resistance, and drought tolerance, to the host plant. *Azospirillum* sp. B510, isolated from rice in japan, can induce disease resistance in rice plants and promote plant growth in paddy field (1, 2). To search more practically useful strain, we exploited these findings and isolated *Azospirillum* sp. strain from field-grown rice. The strain activated the immunity of rice like strain B510. We developed the strain as agricultural material for rice cropping by examination formulation processes. From 2008 to 2010, inoculation experiments with the agricultural material was conducted in Hokkaido, Japan. Stem numbers on panicle formation stage and tiller numbers and seed yield on ripening stage were increased by inoculation with the agricultural material. Similar effects were observed in several areas tested in Japan. Therefore, application of the agricultural material in rice cultivation will be expect to increase crop yield. (1) Yasuda et al. 2009. Bioscience, Biotechnology, and Biochemistry Vol 73, p 2595-2599; (2) Isawa et al. 2010. Microbes and Environts. Vol. 25, p 58-61.

PS05-268

Biocontrol potential of *Bacillus* sp. towards plant pathogenic bacteria from *Dickeya* spp.

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Recently, pectinolytic plant pathogenic bacteria from the *Dickeya* genus, apart of *Pectobacterium* spp., are responsible for the important economic losses in the potato production in Europe.The prevention of the disease spreading is based on hygienic measures and application of certified pathogen-free propagation material. Biological control could be an alternative for standard control management in potato, bringing together the environmentally friendly replacement for chemical and physical control and cost reductions. Bacteria from *Bacillus* genus produce biologically active compounds and are known for their antagonistic properties towards fungal and bacterial plant pathogens. These bacteria are deeply studied for their applicable potential in agriculture.In this study we analyzed the antagonistic potential of 13 *Bacillus* sp. isolates originated from rhizosphere of different plants, towards *Dickeya* spp. These isolates were selected on the basis of their ability to inhibit pathogens growth (7 isolates) or to interfere in quorum-sensing (QS) mechanism mediated by N-acyl homoserine lactones (AHL) (6 isolates). Co-inoculation assay on potato tuber silences confirmed the ability of the 8 isolates to protect plant tissue from the pathogens activity. The AHL-inactivating isolates were active against most of the pathogenic strains tested. It is especially interesting because the QS mechanism seems to be less important in the pathogenicity of *Dickeya* sp. than in *Pectobacterium* genus. The most active isolates were tested for their ability to colonize potato rhizosphere in a growth chamber experiment. For this green fluorescent protein (GFP)-tagged or riphampicine resistant selected isolates were used. Obtained results will be further discussed.

PS05-269

Efficacy of rice stubble degrading microorganisms, fungal antagonist and N-fixing bacterium for enhancing growth and yield of organic rice

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Rice yield is predominated by using chemical fertilizers and pesticides. The health and environmental criteria have led to increase research efforts on alternative methods. The development of organic rice production in this study was emphasized on microbial combinations including rice stubble degrading microorganisms (*Aspergillus* sp., *Azotobacter* sp. and *Saccharomyces cerevisiae*), antagonistic fungus (*Trichoderma* sp.) and N-fixing bacterium (*Bacillus subtilis*) compared to bio-organic liquid applied by farmers. The investigation was carried out at Phayao province, Thailand during July - November, 2011 using RCBD with 3 treatments (bio-organic liquid, microbial combination and non-treated treatments). The result revealed the treatment of microbial combinations showed significantly increase percentages of fresh weight, dry weight, plant shoot, stems per clumps and yield 4.9, 6.5, 3, 9 and 21.8 respectively. Moreover, the soil property of paddy field as organic matter (2.2%), pH (7) and electric conductivity (2.3 mmho/cm) tended to be higher than the control treatment. Especially, total nitrogen in soil significantly increases to 37% after microbial combinations were used. It would be the positive impacts of microbial combinations in producing soil nutrient and organic matter from rice stubble for enhancing growth and yield of rice.

PS06-270

CLE peptide signaling in cyst nematode parasitism

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Plant-parasitic cyst nematodes (*Heterodera* and *Globodera* spp.) are agriculturally-significant pests that cause substantial annual yield losses worldwide. These sedentary endoparasites secrete effector proteins originated from their esophageal gland cells into selected root cells to create a unique feeding cell structure that serves as the sole nutrient source for the nematode to complete its life cycle. Effector proteins sharing similarity to plant CLAVATA3/ESR (CLE) signaling peptides have been identified in several cyst nematode species including soybean cyst nematode (*H. glycines*), beet cyst nematode (*H. schachtii*), and potato cyst nematode (*G. rostochiensis* and *G. pallida*). Plant CLE peptides represent a family of signaling peptides having critical roles in plant growth and development including regulation of stem cell fate in the root meristem. A large body of evidence now supports a role for nematode secreted CLE peptides as ligand mimics of endogenous plant CLE signals to developmentally reprogram the fate of root cells for feeding cell formation. Host plant receptors that interact with nematode secreted CLE signals are being identified by loss-of-function studies and receptor binding assays. These studies have provided new insight into how nematode CLE signals are perceived by host plants to modulate signaling pathways that facilitate the formation of feeding cells within host plant roots. Ultimately, we hope to apply the knowledge of this conserved mechanism of molecular mimicry in nematode parasitism to develop novel forms of engineered resistance in crop plants.

PS06-271

Molecular and functional analysis of rice-nematode interactions

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Our research focuses on rice as model plant to analyse the interaction with nematodes at the cellular and molecular level. To get a comprehensive overview of the compatible plant response to nematode infection, mRNA sequencing was performed on rice after nematode infection. Local infected tissue was compared with systemic tissue after infection by the root knot nematode *Meloidogyne graminicola* or the migratory nematode *Hirschmanniella oryzae* and with control tissue of the same developmental stage. One of the results is the downregulation of plant defense genes locally and systemically after root knot nematode infection. We are also studying the role of several plant hormones in the plants basal defense. For a functional analysis of plant genes that are differentially expressed upon nematode infection, we perform infection experiments on mutants or transgenics with lower or higher expression of that specific plant gene. To get insight in the proteins that are secreted by nematodes into the plant in order to establish a succesful infection, a transcriptome analysis was performed on *Meloidogyne graminicola* preparasitic juveniles and *Hirschmanniella oryzae* mixed stage nematodes. One of the strategies is to identify nematode proteins that are capable of suppressing plant defense. In the future we want to extend our analyses to other types of rice nematodes (cyst nematode, stem nematode, white tip nematode).

PS06-272

Silencing of *Myzus persicae* genes by plant mediated RNAi

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The green peach aphid (*Myzus persicae*) is one of the most significant crop-damaging insect-pests worldwide. Little is understood on how aphids modulate plants into compatible hosts for the aphids and the viruses they transmit. RNA interference (RNAi) is a valuable reverse genetics tool to study gene function in various organisms including aphids. We made use of the *M. persicae* broad plant host range, which includes the model plants *Nicotiana benthamiana* and *Arabidopsis thaliana*, to develop the plant-mediated RNAi technology for aphids. This technology enables aphid gene silencing in the aphid natural environment and minimizes insect handling during experiments (Pitino, Coleman et al, 2011. PLoS One 6: e25709). We targeted *M. persicae Rack1* (*MpRack1*), which is predominantly expressed in the gut, and *M. persicae C002* (*MpC002*), which is predominantly expressed in the salivary glands. The aphids were fed on *N. benthamiana* leaf discs transiently producing dsRNA corresponding to these genes and on *A. thaliana* plants stably producing the dsRNAs. *MpC002* and *MpRack1* expression were knocked down by up to 60% on transgenic *N. benthamiana* and *A. thaliana*. Moreover, silenced *M. persicae* produced less progeny consistent with these genes having essential functions. Similar levels of gene silencing were achieved in our plant-mediated RNAi approach and published silencing methods for aphids. Furthermore, the *N. benthamiana* leaf disc assay can be developed into a screen to assess which genes are essential for aphid survival on plants or for virus transmission. Our results also demonstrate the feasibility of the plant-mediated RNAi approach for aphid/virus control.

PS06-273

Genetical genomics of nematode parasitism

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Root-knot nematodes (RKN: *Meloidogyne* sp.) elicit complex cellular changes in their hosts. Using cross-species, eQTL analysis we ask: how does the genetic make-up of the pathogen influence host gene expression? Specifically, we consider the influence of allelic variation at each nematode locus on the expression of each and every plant gene. Natural genetic and phenotypic variation in field isolates of *M. hapla* has been captured in highly inbred nematode parental lines (VW9 and LM), and 120 recombinant inbred progeny lines (RILs) developed as a mapping population. Replicate pools of Medicago plants have been individually infected with the 120 nematode RILs, and the combined transcriptomes of each individual determined. Mapping the data to the full genome sequences reveals the quantitative expression levels of each plant gene and each pathogen gene. Comparison of VW9 and LM revealed numerous SNPs, including ~14,000 within coding regions; these markers can be scored in RNA-Seq data, permitting each pathogen RIL to be genotyped. Thus far, data from 32 RILs have revealed numerous recombination events, including several apparent hot-spots. Genotyping additional RILs will inform the mapping of QTL and Mendelian loci germane to parasitic ability. Our functional analyses ascribe a parasitic role to several of the complex loci we have identified as encoding mimics of plant peptide hormones (CLE and RAR). Consistent with their roles in parasitism, these loci are highly polymorphic between VW9 and LM; we are interested to see if our cross-species, eQTL approach also will indict these genes.

PS06-274

Functional analysis of root-knot nematode genes and host responses during Arabidopsis infection

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Plant-parasitic nematodes are a huge agricultural problem on many of the world's main food crops, and one of the most damaging of the plant-parasitic nematodes is the root-knot nematode (Meloidogyne spp). These nematodes pose a serious agricultural threat due to their large host range and because many crop plants lack natural nematode resistance. During the susceptible interaction, root-knot nematodes invade host roots where they choose plant cells to convert into metabolically-active feeding sites. The root-knot nematode's manipulation of the plant cell, and in particular how the nematode is able to regulate host plant pathways, is not well-understood. Here we report on the findings from a novel effector screen using a heterologous expression system to functionally analyze the roles of putative root-knot nematode effectors and secreted proteins. By expressing nematode genes in the bacterial pathogen *Pseudomonas syringae* DC3000 and monitoring bacterial growth on infected Arabidopsis leaves, we have tested several root-knot nematode genes. We have discovered that expression of at least one of the nematode genes that we have tested can alter the overall levels of bacterial growth on inoculated Arabidopsis leaves. Interestingly, it lowers bacterial growth, hinting that a protein from a root pathogen has a negative impact on bacterial virulence and growth during leaf infection. In addition, we will briefly discuss plant side of the nematode-plant interaction, with some evidence suggesting that auxin perception is required for full *M. javanica* susceptibility on Arabidopsis roots.

PS06-275

Cowpea aphid, *Aphis craccivora* Koch. feeding behavior and plant antioxidative response in faba bean, *Vicia faba* L. cultivars

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Cowpea aphid, *Aphis craccivora* Koch., performance on five selected faba bean, *Vicia faba* L., cultivars were evaluated through biological study, feeding behavior study (using DC-EPG) and plant antioxidative response. Initial cowpea aphid colony development study ranked the higher resistant from Gazira2>Com.'Misr'>Giza 3Imp.>Goff1>Misr1. Detached leaf biological assay supported the suggested less suitability of Gazira2 compared to Misr1 by having significantly lower net reproduction rate (Ro), intrinsic rate of increase (rm), finite rate of increase (λ), but longer for generation time (T), and doubling time (Td). Feeding behavior study revealed that the different resistant levels among five faba bean cultivars were not due to phloem tissue factors or leaf surface factor, as confirmed by insignificant result of phloem ingestion duration (waveform E2) and scanning electron microscope (SEM), respectively. Resistance factor, especially in Gazira2, is suggested to be due to the longer duration of stylet penetration difficulties (waveform F). Repeated measurement analysis showed there was significantly higher plant antioxidative response activity on Gazira2 compared to Misr1 across the three days of cowpea aphid infestation duration for both peroxidase (POD) and polyphenol oxidase (PPO) activity ($P=0.0005$ and $P= 0.0051$, respectively). Finally, it was suggested that higher activity of POD and PPO in Gazira2 had strong relation with their resistant character of having longer waveform F duration (stylet penetration difficulties).

PS06-276

A natural diterpene as an inducer for resistance to root-knot nematode (*Meloidogyne incognita*) infection in Arabidopsis and tomato

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Plant-parasitic nematodes parasitize roots and/or stems of various host plants, resulting in damage or yield loss. The yield loss due to nematode infection was estimated to reach a hundred million dollars per year. The root-knot nematode (RKN), one of the most devastating pathogenic nematodes, invades plants roots by damaging the root, and the plant should recognize the invasion and transmit the signal to the plant body to defend itself against the attack by RKN. We have recently shown that jasmonic acid (JA), a plant stress hormone, reduces the degree of infection of RKN through activation of defense-related genes in tomato and *Arabidopsis thaliana*. Because JA is known to induce production of various secondary metabolites, we assumed that JA-inducible metabolites would contribute to the reduction in infection. Based on the assumption, we explored RKN infection-inhibiting substances from tomato plants exogenously treated with JA and identified a diterpene as one of such substances. This diterpene reduced RKN infection in tomato and Arabidopsis. The reduction by this compound was altered in several defense-related or phytohormones Arabidopsis mutants.

PS06-277

Unravelling the mechanisms of resistance to bluegreen aphid and pea aphid in the model legume *Medicago truncatula*

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Aphids, including the closely related bluegreen (BGA; *Acyrthosiphon kondoi*) and pea (PA; *A. pisi*) aphid, are important pests in agriculture. Resistance to BGA and PA has been introgressed into the *Medicago truncatula* variety Jemalong (A17) through recurrent backcrosses to create a new aphid-resistant cultivar Jester (91% identical to A17). Resistance to BGA in Jester is conferred by a dominant gene called AKR (*Acyrthosiphon kondoi* resistance) located on the short arm of chromosome three in a region rich in CC-NBS-LRR genes. PA resistance in Jester is conferred by a dominant gene, termed APR (*Acyrthosiphon pisum* resistance), which lies approximately 22.3 cM distal from AKR in a region dense in CC-NBS-LRR genes. Analysis of transcriptional changes in defence related genes representing various signalling pathways and transcription factor profiling showed clear differences in the response to BGA vs. PA.A17 has a moderate resistance to both BGA and PA compared to the highly susceptible accession A20. Quantitative trait loci (QTL) analysis using an A17 x A20 recombinant inbred line population revealed that one locus, which co-segregated with AIN (*Acyrthosiphon* induced necrosis) on chromosome 3, is responsible for the reduction of aphid biomass (indicator of antibiosis) for both PA and BGA, albeit to a lesser degree for PA than BGA. Interestingly, two independent loci on chromosomes 5 and 3 were identified for the plant biomass reduction (indicator of plant tolerance) by PA and BGA, respectively, demonstrating that the plants tolerance response to these two closely related aphid species is distinct.

PS06-278

Application of RNAi to develop plant resistance to nematode pathogens

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The aim of this work is to develop and apply RNA interference (RNAi) technology to establish host resistance in cereal, grass and dicotyledonous crop plants of economic importance. The focus is on resistance to root lesion nematodes (*Pratylenchus* spp.) which reduce yields of wheat, barley and sugarcane crops by 7-15% or more, and the beet cyst nematode (*Heterodera schachtii*), which is a major pest of brassica and beet crops. Using new sequencing technologies we have undertaken transcriptome analyses of *P. thornei, P. zeae*, and *H. schachtii*, and following annotation and comparative genomic analyses (Nicol et al. Int. J. Parasitol. 42, 225-237, 2012), a series of potential target genes were identified which if silenced would confer host resistance. Two approaches to test the effects of silencing these target genes were undertaken: soaking J2 nematodes in dsRNA, and delivery of dsRNA to nematodes via transgenic plants. Methods were established to generate transgenic plants of wheat, sugarcane and Arabidopsis, and for analysis of RLNs after soaking experiments. Replicated lines of different transgenic events were challenged in soil or in sand with J2s of *H. schachtii* (Arabidopsis) and mixed stages of RLNs (wheat and sugarcane) of the different nematode species. With reductions in nematode replication of up to 90% or more, the results provide clear proof-of-concept that RNAi can be used to confer host resistance to nematode pathogens both in dicotyledonous and monocotyledonous crop plants.

PS06-279

Unravelling the molecular events involved during the early pathogenic interaction between *Meloidogyne incognita* and *Arabidopsis thaliana*

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The root knot nematode *Meloidogyne incognita* is an obligate parasite that can infect up to 1700 plant species. These nematodes penetrate the root at the elongation zone and then migrate to the zone of differentiation where they establish their feeding site. Although physiological and molecular changes inside the root leading to the feeding site formation have been widely studied, very little is known about the molecular events preceding root penetration by nematodes. However understanding how nematodes successfully penetrate their host could lead to novel control strategies. This work aims to understand the early signalling and molecular events involved before and during *M. incognita* penetration of *Arabidopsis thaliana* roots. As a preliminary screen, we used sterile root exudates from *A. thaliana* to study *M. incognita* behaviour and gene expression. We show that the nematodes are able to perceive and respond to the root signals. Based on this work and using next generation RNA sequencing we are now analysing the nematode and plant transcriptome during very early interaction stages, compared to non-infective nematodes and mock-inoculated roots. With the objective to identify new nematode effectors, we are also using an *in silico* approach to predict the nematode protein secretome and identify those genes highly expressed during the early plant infection.

PS06-280

Involvement of plant CLE peptide signaling in nematode infection process in tomato

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CLE peptide hormone is the molecule responsible for the control of plant meristem activity, and CLE genes are conserved in many plants. On the other hand, in the animal kingdom, only nematode that is infective to plants has CLE gene. This CLE gene of nematode is likely to be functional in the plants. Once nematodes infect roots, nematodes make root cells into the multinucleated giant cells, as source of nutrition, by injection of various redifferentiation factors thought to be involved CLE peptides of nematodes. In this study, to elucidate the molecular mechanism of the infection process of nematode, nematode infection experiments to plants were performed. Lots of wild-type strains and cultivars of tomato were infected by Nematode (*Meloidogyne incognita*). Compared with infection rate as an index of root-knot number, it was revealed that Micro TOM (*Solanum lycopersicum*), *Solanum pennellii*, *Solanum peruveanum* showed resistance to the nematode infection. Following that, we identified and analyzed the sequences of tomato homologue genes of *CLV2, RPK2, SOL2*, and *CLV1*, which are involved in CLE peptide signaling of Arabidopsis. There are some SNPs in these genes.

PS07-281

Functional analysis of *Xanthomonas campestris* pv. *campestris* type III effectors using transgenic plant approach

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The black rot disease caused by *Xanthomonas campestris* pv. *campestris* is one of the most important disease in cruciferous crops. Because the virulence of *X. campestris* pv. *campestris* is dependent on the type III secretion system (T3SS) to inject effector proteins, it is important to study the interaction between plant and pathogen through type III effectors for controlling Xcc disease. Here, 13 type III effectors of *X. campestris* pv. *campestris* 8004 were analyzed by transgenic Arabidopsis approach. Among them, XVE::AvrXccC8004, XVE::XopAC8004, XVE::XopX8004 and XVE::AvrBs18004 transgenic lines show cell death phenotype; XVE::XopD8004 and XVE::XopP8004 show abnormal development phenotype. In order to understand the mechanism of cell death induced by AvrXccC8004, transgenic lines expressed truncated fragments of AvrXccC8004 were created. Through trypan blue staining and TEM analysis, we found that the AvrB_AvrC domain alone can trigger cell death. For RNA-seq analysis on XVE:AvrXccC8004 (110-440 a.a.) transgenic plants, 24.9 million reads were obtained from DMSO- and estradiol-treated samples. After DESeq analysis, a total of 381 unique genes with $p < 0.01$ were identified as differential expressed genes. Among them, 111 out of 381 differentially expressed genes were mapped to response to stimulus term by Gene Ontology (GO) analysis which suggested that Arabidopsis could response to the activities of the AvrB_AvrC domain of AvrXccC8004 and regulated genes expression for cellular or behavioral stimulations.

PS07-282

Identification of infection stage-specific effector molecules of the Asian soybean rust fungus *Phakopsora pachyrhizi*

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Asian soybean rust (*Phakopsora pachyrhizi*) causes severe yield losses in soybean growing regions in North- and South-America. Since soybean plants with resistance to all isolates of the pathogen are not yet available, and fungicidal treatments are profit-decreasing, soybean cultivation in areas invaded by ASR might become limited. Facing this scenario we followed a knowledge-based approach by deducing novel strategies to combat the disease from detailed investigations of the fungal infection process. ASR pursues a Janus-faced infection strategy by killing the penetrated epidermal cells which is atypical for an intrinsic biotrophic pathogen. Later on ASR establishes an ordinary biotrophic interaction by forming haustoria inside mesophyll cells. Besides serving as feeding organs, haustoria also secrete effectors that may interfere with the host's defence machinery. Aiming at the identification of this secretome, we targeted the haustorial transcriptome by a next generation sequencing approach using RNA of isolated haustoria in comparison to RNA extracted from infection structures including appressoria. After de-novo assembly and annotation, we performed an in silico screen for genes encoding for putatively secreted proteins. For the functional analysis of candidate genes in ASR, we are working towards the establishment of a transient host-induced gene silencing assay. In this way silencing of fungal genes could be achieved by expressing the respective interfering RNAs in the host tissue. Data on the gene silencing mechanism in ASR using artificial siRNA and in planta expressed hairpin constructs, as well as virus induced gene silencing, will be presented.

PS07-283

***Pectobacterium carotovorum* uses the type III secretion machinery to suppress systemic defense in host plants**

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We show that delivery of the type III secretion system (T3SS) effector protein DspE to plant cells by *Pectobacterium carotovorum* leads to strong local induction of known plant defense markers including *PR* and phytoalexin biosynthesis genes in both potato and tomato plants. However, in potato tubers the expected systemic induction was not observed at least for some of these genes. Inactivation of the key regulatory (HrpL) or structural (HrcV) component or the main effector (DspE) of the type III machinery in *P. carotovorum* resulted in systemic induction of genes weakly induced (or not induced at all) by the wild type bacterium. We have also observed significantly reduced maceration of potato tuber tissue by the T3SS mutants compared to the wild type bacteria. We suggest that T3SS may provoke rapid death of the cells close to the infection site which may be advantageous for the necrotrophic pathogen and may not allow enough time for the proper induction of systemic defense reactions.

PS07-284

A rice blast fungus alpha-L-arabinofuranosidase protein MoABFb is related with *Magnaporthe oryzae* infection in rice

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Glycosyl hydrolase family protein is a widespread group enzymes that hydrolase the glycosidic bond between two or more carbohydrates. During host infection process, *M. oryzae* secreted out a series of GH family proteins to degrade rice wall for successful infection. One of those GH family protein alpha-L-arabinofuranosidase B (MoABFb), which belong to the GH43 subfamily, was previously detected related with fungal infection process. Sequence alignment of MoABFb homologs revealed a high conservation of amino acid sequence with GH43 family proteins from other species. Biochemical analysis by using recombinant MoABFb protein confirmed that MoABFb contains a high arabinofuranosidase activity. SEM data indicated that the expression of MoABFb is related with host cell wall degradation. RT-PCR and leaf blot results suggested that MoABFb was accumulated after 48 h after inoculation to the compatible rice strain and revealed a good match with fungal infected pattern. In vitro and in vivo cell death assay using cell death reporter *PBZ1 pro::GFP* indicates that expression of MoABFb related with cell death activation in host. To understand the role of MoABFb in fungal pathogenicity, gene deficient and over-expression mutants were generated. The deficient mutant increased fungal susceptibility, and over-expression mutant reduced the infection pattern, suggesting that MoABFb related with fungal pathogenicity

PS07-285

A *Magnaporthe oryzae* secreted effector, MoCP, activates host autophagic programmed cell death

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The rice blast fungus secreted protein MoCP belongs to the cerato-plantanin family, which has phytotoxic activity against various plants. Here, we characterized that MoCP protein induces programmed cell death (PCD) in rice. ROS accumulation, ion leakage, DNA fragmentation, cytochrome c release, chromosome

shrinkage, nuclear condensation, and autophagy formation, which were cell death markers, were investigated using biochemical and histochemical approaches. PCD was induced in rice suspension cultured cells after treatment with exogenous MoCP under time and dosage dependent manner. Furthermore, exogenous MoCP protein induced defense responses such as PR genes and MAP kinase activation in rice. MoCP with or without signal peptide derived from rice secreted protein glucanase was fused with mCherry reporter, and then overexpressed in cell death inducible promoter transgenic plant, PBZ1pro::GFP. Only the secreted protein activated GFP signal and induced defense related gene expression. These results indicated that MoCP may active cell death after secreted out of plasma membrane but not in the cytoplasm, and activates host autophagic PCD.

PS07-286

The biotroph *Phakopsora pachyrhizi* pretends a necrotrophic pest

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Phakopsora pachyrhizi is a biotrophic fungus causing Asian soy rust disease. On both its soybean host and Arabidopsis nonhost *P. pachyrhizi* directly penetrates epidermal cells that then commit cell suicide. The hypersensitive response coincides with activated expression of *PDF1.2*, a marker gene for defense to necrotrophic pests. We would like to elucidate whether *P. pachyrhizi* actively affects nonhost gene expression prior to penetration. By application of cell-free germination fluids onto Arabidopsis leaves we demonstrated that *PDF1.2* expression does not depend on the presence of fungal structures or penetration. This finding supports our hypothesis that *P. pachyrhizi* mimics at least some aspects of a necrotroph to disguise its biotrophic nature. By fractionating *P. pachyrhizi* germination fluid it was shown that *PDF1.2* eliciting activity resides in a proteinaceous fraction. Further analyses will be done to identify the responsible proteins.

PS07-287

Effector CoDN3 of *Colletotrichum orbiculare* suppresses NIS1-induced cell death of *Nicotiana benthamiana*

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Colletotrichum orbiculare (*Co*), the causal agent of cucumber anthracnose, infects *Nicotiana benthamiana* (*Nb*). Transient expression of the secreted protein gene *NIS1* by agro-infiltration induces cell death in *Nb* (NIS1-induced cell death, NCD). Promoter assay using GFP suggested that *NIS1* is preferentially expressed in invasive biotrophic hyphae of *Co*. This has led to a proposal that NCD occurs in *Nb* at the infection process of *Co*. However, the knockout mutants of *NIS1* showed normal virulence on *Nb*, suggesting a possibility that NCD is suppressed in *Nb* during *Co* infection. Previously, *CgDN3*, encoding a secreted small protein, was identified as a pathogenicity-related gene of *C. gloeosporioides* (*Cg*) that infects *Stylosanthes guianensis*. The knockout mutants of *CgDN3* elicited a local hypersensitive-like response by the host plant, implying the ability of CgDN3 to suppress the hypersensitive-like cell death. We identified a homologue of *CgDN3* in *Co*, designated *CoDN3*, and investigated its suppressive effect on NCD. As a result, we found that CoDN3 is an effector functioning as a suppressor of NCD. Furthermore, to elucidate CoDN3 function as a secreted effector in detail, we visualized CoDN3 protein fused with mCherry during host plant invasion and detected the highly concentrated signal of CoDN3 at the neck region of biotrophic invasive hyphae beneath the appressorial penetration site, implying vigorous secretion of effector at this point. We will present detailed analysis on the effector-concentrated zone using multiple cellular markers. [This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences.]

PS07-288

The structure and evolution of barley powdery mildew effector candidates

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The powdery mildew fungus, *Blumeria graminis f.sp. hordei* (Bgh) is a recently sequenced (Spanu et al. 2010), obligate biotrophic pathogen of barley with a significant agricultural impact and serve as a model for studies on powdery mildews and other obligate biotrophic interactions. Here I present a comprehensive survey of the 491 Candidates for Secreted Effector Proteins (CSEPs) representing more than 7% of the protein coding genes found in the Bgh genome. Based on sequence homologies we clustered the CSEPs into families of paralogs and show that CSEP genes have duplicated in the genome most likely due to unequal crossing over during evolution and they are therefore clustered in the genome. Within many of these families we find strong evidence for positive selection for diversity. When we mapped the amino acid residues under positive selection on 3D structural models they were usually predicted to be exposed and thus possibly involved in protein interactions. Expression studies show that the CSEPs preferentially are expressed in haustoria.Many CSEPs from different families appear to be related to microbial RNAses and we propose that a large proportion of the CSEPs have evolved from an ancestral microbial RNAse. We speculate that these RNases may have been an ideal starting material for building up an effector arsenal. Our data fit well with a model for CSEP evolution driven by selection for gene duplications and selection for amino acid chances resulting in a large diversity allowing the fungus to yield a varied palette of effectors functions.

PS07-289

Dissecting functions of *Hyaloperonospora arabidopsidis* effectors by transcriptome approach

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Oomycete pathogens secrete effector molecules to attenuate plant defense signaling during colonization of their hosts. We have revealed that candidate effectors (HaRxLs) found in the genome sequence of Arabidopsis downy mildew (*Hyaloperonospora arabidopsidis-Hpa*; Baxter et al. 2010 SCIENCE) could positively contribute to bacterial virulence and suppress PAMP-triggered immunity (Fabro et al. 2011 PLOS Pathogens). Also, the subcellular localization of HaRxLs *in planta* was investigated (Caillaud et al. 2012 Plant J.) and putative plant targets of HaRxLs were identified by yeast two hybrid screening (Mukhtar et al. 2011 SCIENCE). However, the mechanisms by which *Hpa* effectors promote virulence remain to be elucidated. We have established a robust expression profiling method, named Gene Expression Profiling through Random Sheared cDNA tag sequencing (EXPRSS), which could detect differential expression with higher sensitivity than microarray method. To identify the effector virulence functions, transcriptome analysis using EXPRSS was carried out on lines that express several effectors targeted to the host cell nucleus, which we would expect to interfere with transcriptional regulation of defense

genes in infected host cells. Putative targets and functions of *Hpa* effectors are discussed on the basis of transcriptome data.

PS07-290

OsPUB44, a regulator of PAMPs-induced basal resistance, is targeted by type III effector Xoo3222

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Many gram-negative bacteria that infect plants directly inject lots of effector proteins into host cells using type III secretion system (TTSS). The type III effector proteins are considered to be the primary virulence factors and strongly contribute to cause disease on the host plants. Therefore, it is likely that these effectors block the important steps in plant immune response. Xoo3222 is one of effector proteins secreted into rice cells through the TTSS of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). To elucidate the physiology of Xoo3222, we have generated transgenic rice plants expressing *Xoo3222*. The transgenic plants showed an enhanced susceptibility to the TTSS-deficient *Xoo* mutant. Additionally, microarray experiments revealed that Xoo3222 strongly affected expression of a large number of PR genes induced by the chitin elicitor, suggesting that Xoo3222 may target host factors that function in PAMPs-triggered immunity in rice. We identified OsPUB44 as an interactor of Xoo3222. OsPUB44 encodes ubiquitin E3 ligase with U-box and ARM domains. Expression levels of OsPUB44 were increased by the chitin elicitor treatment. Xoo3222 specifically interacted with the U-box domain of OsPUB44. Furthermore, OsPUB44 possessed the ubiquitin E3 ligase activity *in vitro*, which was inhibited by Xoo3222. Interestingly, Xoo3222 did not interact with the OsPUB44 homologous proteins. The two hybrid experiments showed that three amino acid residues within the U-box motif of OsPUB44, which are distinct from those of OsPUB44 homologous proteins, are responsible for interaction with Xoo3222. These findings suggest that Xoo3222 may inhibit rice innate immunity via specific modulation of OsPUB44 activity.

PS07-291

Biogenesis of sRNAs homologous to effector-encoding genes and transposable elements in *P. infestans*

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We have prepared small RNAs from mycelia, sporangia, germinating sporangia and germinating cysts of two contrasting isolates (Vetukuri et al. 2012). Deep sequencing of small RNAs from eight libraries generated 15.3 and 12.8 million high-quality sequence-reads, respectively. Total reads were individually mapped to datasets of RXLR and CRN effectors, transposons and the whole genome, after filtering reads that matched to tRNAs, rRNAs and mtDNA. Alignment of all sequences to the entire P. infestans genome sequence revealed an enrichment of 21, 25, 26 nt sRNAs and in the range of 30-33 nt. The highest proportions of sRNA sequences were homologous to transposons, followed by CRN and RXLR effectors. The most striking accumulation of sRNA sequences was observed for CRN genes, where the majority of sRNA sequences were 21 nt in length. 5' nucleaotide preference of sRNAs differs between RNAs mapped to transposons, RXLRs and CRN leading to the suggestions that different Argonautes might process different sRNAs. Via PiDcl1-eGFP fusions we found DCL1 localized to the cell nucleus in P. infestans. Analysis of the 5' base of the total sRNAs mapping to transposons, RXLR and unplaced reads of the genome showed different preferences, and suggests that different processes are used for the biogenesis of each size class of sRNA. But 3' ends of small RNAs are not modified. 7 miRNAs, precursors and target genes have been predicted. A selection of transposons, and genes encoding RXLR and CRN effectors with a high abundance of homologous sRNAs were analyzed in more detail.

PS07-292

Functional analysis of the tumor and anthocyanin-inducing effector protein Tin2 of *Ustilago maydis*

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The fungus *Ustilago maydis* is the causal agent of smut disease in maize. The interaction with the host is governed by secreted effectors and many of the respective genes reside in clusters in the genome. Cluster19A is the largest of these clusters carrying 24 genes for putatively secreted effector proteins. Deletion mutants of the left half of cluster 19A (19A_1) show dramatic reduction of tumor formation and loss of anthocyanin induction, which are characteristic phenotypes of maize leaves infected with *U. maydis*. We demonstrate that Tin2 effector encoded in this region is secreted and expressed exclusively during biotrophic growth. *tin2* deletion mutants showed small reduction of tumor formation and loss of anthocyanin induction. Introduction of the *tin2* gene into the 19A_1 mutant partially rescued tumor formation and fully restored anthocyanin induction. A Tin2 protein lacking the C-terminal 5 amino acids had lost these abilities. In line with this, Tin2 mutant protein could not interact with cytoplasmic maize protein kinase ZmTTK1 identified as Tin2 interactor by yeast two hybrid screening. Transient expression assays in *Nicotiana benthamiana* revealed that ZmTTK1 was degraded proteasome-dependently. Interestingly, co-expression with Tin2 stabilized ZmTTK1. Tin2-binding region of ZmTTK1 contains the phosphodegron-like motif DSGxS. When ZmTTK1 carrying mutations in this motif was transiently expressed, the mutant protein proved more stable than the wild type protein. Therefore, it is likely that Tin2 effector masks the phosphodegron motif of ZmTTK1, which stabilizes functional full-length ZmTTK1 kinase in plant cell, resulting in signal transduction leading to anthocyanin biosynthesis and tumor induction.

PS07-293

Horizontal transfer of *holPsyAE* TTSS effector gene in *Xanthomonas campestris* strains

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Xanthomonads are pathogens of many important agricultural plants. Pathogenicity of many Gram-negative bacteria is depended on the type III secretion system (TTSS), which transport at least 30 virulence effector proteins into plant cell, where they subvert the host cell physiology and disrupt host defense mechanisms. The fact that homologous effector genes were found in different species and even genera of plant-associated bacteria was explained by frequent horizontal transfer of these genes. Presence of mobile genetic elements in vicinity of several effector genes indirectly confirms the hypothesis. We have investigated genetic diversity of 30 TTSS effector genes in a population of *Xanthomonas campestris* field strains in order to evaluate possible horizontal transfer of the

effector genes across the genus *Xanthomonas*. In a few strains of *X. campestris pv. campestris* we have found conservative region (318bp) which is homologous to *holPsyAE* from *X. oryzae pv. oryzae*. Assuming the presence of homologous genes, we have analyzed this region and found that these strains have a gene homologous to *holPsyAE* at 97%. After sequencing this region we have identified conservative DNA fragments likely to be associated with genetic transfer and insertion of this gene. We are investigating possible protein-protein interactions of this homolog *holPsyAE* with *Arabidopsis* proteins. This gene is probably important to understanding of plant-microbe interactions, because of its high sequence identity in the remote species of the genus *Xanthomonas*. This work was supported by grants 10-04-01195-a RFFI, "Living nature: current state and developmental problems" from Program of RAN Presidium and ISTC #3431.

PS07-294

Arabidopsis powdery mildew effector proteins target highly connected host proteins and display virulence activity

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Microbial plant pathogens employ a diverse set of effector molecules to manipulate the host cell during infections. While the effector complement of pathogenic bacteria has been mostly elucidated, the repertoire and host targets of fungal effectors are currently underexplored. Here, we characterize the haustorial effector complement of the Ascomycete *Golovinomyces orontii*, the causal agent of the powdery mildew disease in *Arabidopsis thaliana*. From a haustorial cDNA library, we have identified transcripts coding for 120 candidate secreted proteins and were able to obtain full length clones for 84 of these. Transcription profiling of selected effectors suggests their sequential delivery during pathogenesis. In order to explore the interactome of the cloned effector complement we conducted a high-throughput yeast two-hybrid screen against a library of Arabidopsis full length Open Reading Frames. This approach yielded 132 high quality interactions between 47 effectors and 61 corresponding plant proteins. Interestingly, we found a large overlap with the effector-plant interactomes of the bacterium *Pseudomonas syringae* and the oomycete *Hyaloperonospora arabidopsidis*. Proteins targeted by all three pathogens are highly connected in the Arabidopsis interactome. Mutant lines of these genes display disease phenotypes, suggesting that evolutionary distinct plant parasites target the same conserved hubs to promote virulence. Six effectors also enhance virulence of effector-delivering bacteria and suppress induced cell death in *Nicotiana benthamiana*. Their Arabidopsis protein targets are currently being characterized in more detail.

PS07-295

***Xanthomonas campestris* Type III effector XopJ targets the host cell proteasome to suppress plant defence**

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Xanthomonas campestris pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease in pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*). To overcome the basal defence of plants *Xcv* translocates about 30 effector proteins via its type III secretion system into the host cell. XopJ is a *Xcv* type III effector protein grouped into the YopJ/AvrRxv family of SUMO proteases/acetyltransferases although its biochemical activity has not yet been demonstrated. In this study, we characterise the virulence function of XopJ. The effector protein is localised to the plasma membrane via posttranslational modifications involving myristoylation and most likely additional palmitoylation. It subverts basal defence responses by inhibiting vesicular trafficking to the plasma membrane and as a consequence of that also papillae associated deposition of callose. Secretion assays using a secretable GFP reporter protein showed that the inhibition of protein secretion requires an intact catalytic triad and plasma membrane localisation involving myristoylation. Using the yeast two hybrid system and in planta BiFC assays we were able to identify RPT6, a subunit of the 26Sproteasome, as a binding partner of XopJ. Mutational analysis showed that the interaction between XopJ and RPT6 required an intact catalytic triad which suggests that its biochemical activity is necessary for XopJ to bind RPT6. Biochemical studies indicated that expression of XopJ in leaves of *Nicotiana benthamiana* dramatically increases the amount of ubiquinated proteins and significantly reduces proteasome activity. Thus, XopJ might contribute to bacterial virulence by inhibiting proteasome activity through interference with RPT6 function.

PS07-296

Functional characterization of small, cysteine-rich secreted effectors from the filamentous fungus *Magnaporthe oryzae*

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The filamentous fungus *Magnaporthe oryzae* is the most destructive pathogen of rice worldwide. It is described as having two distinct lifestyles within the host plant: a biotrophic interaction during the early stages of infection followed by a necrotrophic phase characterized by host cell death and lesion formation. To identify effector proteins that contribute to pathogenesis, the genome of *M. oryzae* strain 70-15 was mined for proteins that contain a signal peptide, have greater than 3% cysteine content, and are less than 250 amino acids in length. These criteria were selected based upon the characteristics of known effectors from other plant-pathogenic fungi and oomycetes. These proteins were then transiently expressed in *Nicotiana benthamiana* leaves via agroinfiltration to determine if the putative effectors can elicit cell death and thus potentially play a role in the necrotrophic phase of infection. Of the 70 candidate effectors tested to date, 10 were found to induce necrosis and are being evaluated for necrosis inducing activity in barley. In addition, candidate effectors are being co-agroinfiltrated with the BAX gene, a known inducer of host cell death in both plant and mammalian cells. Candidate effectors that suppress the necrosis inducing activity of BAX are potentially involved in suppressing host plant defenses and could contribute to the biotrophic phase of infection. Furthermore, the genomes of 40 *M. oryzae* isolates are being analyzed for candidate effectors to identify those that are conserved amongst some or all isolates and those that are isolate specific to better define their role in virulence.

PS07-297

***Xanthomonas* T3S effector XopX suppresses effector triggered immunity to promote pathogenesis**

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Type III secretion effectors (T3Es) of plant pathogenic bacteria are translocated directly into the host cell cytoplasm during infection and have been shown to suppress immune signaling and defense. The *Xanthomonas campestris vesicatoria* (Xcv) T3E XopX is an virulence factor that appears to suppress host defense, but details of XopX activity and potential host targets are unknown.

XopX is a 699-amino acid protein conserved among most known *Xanthomonas* strains. Highly conserved motifs among XopX alleles include an N-terminal alanine rich region and a predicted tyrosine phosporylation site at amino acid 275. XopX alleles are also homologous to the Xanthomonas T3E Early Chlorosis Factor (ECF). The closest XopX homolog in Pseudomonas syringae is HopAE1. We demonstrated that XopX suppresses the hypersensitive response (HR) elicited by Xcv in the non-host plant *Nicotiana tabacum*. We performed an Agrobacterium-mediated transient expression screen of an Xcv T3E library to identify T3Es eliciting HR in *N. tabacum*. Finally, we demonstrated that an Xcv *xopX* mutant strain elicits an aggravated HR and has reduced growth in the natural host, tomato. Future work will determine whether XopX activity is specific to effector triggered immunity. We will perform a structure-function analysis of XopX to identify domain(s) and residue(s) responsible for its activity and identify host proteins that interact with XopX through protein-protein interaction analyses.

PS07-298

Screening of *Ralstonia solanacearum* effectors suppressing host immune responses

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R. solanacearum is type of gram negative bacteria and the causal agent of bacterial wilt, not only in *Solanaceae*, but also in more than 200 other plant species. Initial plant-pathogen interaction is a critically important for determination of *R. solanacearum* infection. For successful infection, bacterial pathogens inject type III effectors into host cells and inhibit host MTI (MAMP-Triggered Immunity) or ETI (Effector-Triggered Immunity). More than 45 effectors have been predicted in the *R. solanacearum* genome. However, only a few effectors that suppress host immune responses have been identified.We collected 92 putative effectors from the genome information of *R. solanacearum* GMI1000 and the literature-based knowledge. We examined them to find effectors which have an ability to suppress HR (Hypersensitive Response) like cell death. For this purpose, we coexpressed each effector with either INF1 or MEK2^{DD} to induce HR like cell death by agroinfiltration in *Nicotiana benthamiana* leaves. We will discuss possible functions of positive effectors from ongoing screening.

PS07-299

Manipulation of plant immunity signalling by the Late Blight RXLR-WY effector PexRD2

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Oomycetes of the genus *Phytophthora* represent some of the most destructive pathogens of crop species, causing major yield and economic losses worldwide. *Phytophthora infestans*, the causative agent of late blight, is a devastating pathogen of potatoes and tomatoes. To colonise host plants, it secretes an arsenal of effector proteins that are thought to contribute to virulence by suppressing the plant immune system. *Phytophthora* effectors from the RXLR effector family translocate inside host cells to interact with their host targets and modulate host cell function. The targets of a number of these RXLR effectors are now beginning to be identified. It is hoped that by gaining insights into these effector-target interactions - the molecular frontline of the co-evolutionary arms race between the pathogen and host - will eventually lead to novel strategies to control crop diseases. We have used a Y2H screen to reveal that PexRD2, an RXLR-WY effector, interacts with a multi-domain host protein implicated in the cell death signalling pathways associated with plant immunity. We have also shown that ectopic expression of PexRD2 enhances the growth of the pathogen in a model host, *Nicotiana benthamiana*, and suppresses the hypersensitive cell death associated with the recognition of a number of avirulence proteins.

PS07-300

Effector Avr-Pita may form complex with Pi-ta and COX11 in the mitochondria and modulate ROS production

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Avr-Pita/Pi-ta is a classic pathosystem to study the mechanisms of molecular interaction between blast fungus (*Magnaporthe grisea*) and cereal plant rice (*Oryza sativa* L.). Both effector and resistance genes have been cloned for more than a decade, while the in vivo recognition processes of Avr-Pita and Pi-ta are still largely unknown. In current study, we fused a GFP or an YFP fluorescent protein to Pi-ta and Avr-Pita respectively and demonstrated that effector Avr-Pita is colocalized with cognate Pi-ta protein in the mitochondria. Then, a nuclear encoded mitochondrial protein COX11 was identified as one of Avr-Pita-interacting components in yeast two-hybrid assays. We further demonstrated that COX11 is a negative regulator of reactive oxygen species (ROS) accumulation. Taken together, we reason that some fungal pathogens deliver effector protein into the mitochondria of host. And the effector may enhance the function of COX11 by physical interaction to eliminate ROS. While cognate R protein arrests the effector and COX11 to boot up ROS production leading to plant innate immune responses.

PS07-301

Families of candidate effector proteins identified from the haustorial transcriptomes of *Uromyces appendiculatus* and *Phakopsora pachyrhizi*

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Rust fungi are biotrophic pathogens, which do not kill their respective host plants but are dependent on living tissue for propagation. Among them are species with major economic impact like *Phakopsora pachyrhizi* and *Uromyces appendiculatus*, infecting soybean and common bean. A hallmark feature of biotrophic fungi are haustoria, which are the interface for nutrient uptake in rust fungi and probably also for transfer of effector proteins into the plant cell. We did transcriptome sequencing of RNA from isolated haustoria of both *U. appendiculatus* and *P. pachyrhizi* using the next generation sequencing technology 454 pyrosequencing. Comparing our annotation results with those for pre-biotrophic structures we could corroborate findings that haustoria have indeed important functions in energy and amino acid metabolism. BLASTing our sequences against selected rust and basidiomycete genome sequences, predicting secreted proteins and building gene families through clustering, we could identify genes and gene families that are secreted and that are specific to rust fungi or subclades to the rust fungi. Families specific to the legume host seem to be missing but we found at least one family that seems to be present in pathogens in general. In addition to phylogenetic distribution interesting motifs and expression patterns make these genes and gene families good candidate effectors. We are now using phenotypic assays and identification of interaction partners to confirm and characterize effector proteins.

PS07-302

Analysis of defense-associated MIN7 protein complex in Arabidopsis

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Plants have evolved a powerful immune system to defend against infection by most microbial organisms. However, successful pathogens, such as Pseudomonas syringae, have developed countermeasures and inject virulence proteins into the host cell to suppress plant immunity and cause diseases. During P. s. pv. tomato (Pst) DC3000 infection of Arabidopsis, the host ARF GEF protein MIN7 is destabilized, via the host 26S proteasome, by the pathogen effector HopM1. We found that MIN7 has a broad role in pathogen-associated molecular pattern (PAMP)-, salicylic acid (SA)-, and effector-triggered immunity. The MIN7 level in healthy plants is low, but increases posttranscriptionally in response to the activation of defense. Live cell imaging shows that HopM1 acts within the trans-Golgi network/early endosome of plant cells to destabilize MIN7 during Pst DC3000 infection. Native polyacrylamide gel electrophoresis analysis showed that MIN7 exists as an over 500 kDa protein complex in leaf cells. To identify the MIN7 interactor protein(s), we performed co-immunoprecipitation. MIN7 was purified from transgenic Arabidopsis plants expressing MIN7-GFP after treatment with benzothiadiazole to induce SA-dependent immunity. Mass spectrometry analysis indicated that four proteins (the ARF GTPase ARFA1, the ARF GEF BIG2, the 14-3-3 protein GRF1, and a tetratricopeptide repeat (TPR)-like superfamily protein). TPR-like superfamily protein SALK KO line (tpr-1) shows similar phenotypes to min7 such as early senescence under stressed condition. We are testing the hypothesis that ARFA1, BIG2, GRF1, and/or TPR-like superfamily protein are required for MIN7-mediated defense.

PS07-303

New races with unique mutations in avirulence genes overcoming tomato *Cf* resistance genes in a Japanese population of *Cladosporium fulvum*

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Leaf mold of tomato is caused by the biotrophic fungus *Cladosporium fulvum* which complies with the gene-for-gene system indicating that each dominant pathogen avirulence (*Avr*) gene product is recognized by the product of a corresponding dominant host C. fulvum (*Cf*) resistance gene. As a result of selection pressure imposed by *Cf* genes often, pathogenic races developed adapted to the introduced *Cf* resistance genes. The fungus has been reported to occur on tomato in Japan since the 1920s. Initially only race 0, unable to overcome any of the known *Cf* genes, was reported. However, during the last two decades *Cf* resistance genes have been introduced and new races evolved adapted to corresponding *Cf* genes. Here we determined the virulence spectrum of 123 *C. fulvum* strains collected from different parts of Japan and sequenced their avirulence (*Avr*) genes to get detailed information on the molecular basis of adaptation to the different *Cf* genes. Ten races of *C. fulvum* were identified of which races 9, 2.9, 4.9 and 4.9.11 occur in Japan only. The *Avr* genes of these races contain unique mutations causing adaptation to *Cf* genes including (i) frameshift mutations and (ii) transposon insertions in *Avr2*, (iii) point mutations in *Avr4* and *Avr4E*, and (iv) deletion of *Avr4E* and *Avr9*. It is concluded that molecular mechanisms of adaptation to different *Cf* genes in an isolated *C. fulvum* population in Japan are unique but follow similar patterns as those observed in other parts of the world.

PS07-304

Functional studies of *Pseudomonas syringae* type III effector AvrE

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To subvert plant immunity, Gram-negative phytopathogenic bacteria inject numerous effector proteins into the plant cell. The AvrE family of type III effectors is broadly conserved and very important for pathogenesis. However, the molecular mechanisms of their virulence functions are not understood. We are using Arabidopsis-*Pseudomonas syringae* pv. *tomato* (Pst) DC3000 pathosystem to study AvrE function. We identified two sequence motifs in AvrE: a WxxxE motif within the N-terminal half and a KK motif at the C-terminus. When transgenically produced in Arabidopsis, wild-type AvrE complemented the growth of the Pst DC3000 deltaCEL mutant, in which four conserved effectors including avrE are deleted. In contrast, transgenically expressed WxxxE or KK motif mutants lost the ability to complement the deltaCEL mutant, demonstrating the WxxxE and KK motifs are essential for AvrE function. Remarkably, we recently found that the WxxxE motif can be relocated to four other tryptophan (W) positions where these Ws are conserved among AvrE-family proteins. When co-expressed in tobacco plants, the N- and C-terminal halves of AvrE can reconstitute the cell death-inducing activity of wild type AvrE and the two half proteins interact, as demonstrated by co-immunoprecipitation, suggesting AvrE is a modular protein and contains at least two functional domains. Using fluorescence protein tagging, AvrE protein is found to be localized on the plasma membrane and form speckles. Yeast two-hybrid and in planta pull down using AvrE as bait revealed several candidates of AvrE interacting proteins, which paved a way for identifying molecular targets of AvrE in the plant cell.

PS07-305

Identification of effectors from *Blumeria graminis* by *Xanthomonas* type three secretion and virus-induced gene silencing based screens

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Identification and characterization of *Blumeria graminis* effectors is critical for obtaining a better understanding of barley powdery mildew interactions, which may contribute to new control strategies. By proteomics, we identified about 40 *Blumeria* effector candidate (BEC) genes from the recently completed *Blumeria graminis* genome sequence. Host-induced silencing of these genes with particle bombardment revealed nine that appear to play a role in the host-pathogen interaction. We fused each of the coding sequences to the 5· end of the avrBs2 effector gene from *Xanthomonas campestris* for bacterial type III delivery into barley as well as maize and rice. When delivered into barley by a strain of *X. campestris* that otherwise elicits no visible plant response, one of the candidates, BEC1019, suppressed a defense-associated cell death elicited by another, co-inoculated, *Xanthomonas* species, *X. oryzae*. Meanwhile, each of the nine BECs was also silenced by *Barley stripe mosaic virus* (BSMV) induced gene silencing and followed by inoculation of *Blumeria graminis* isolate 5874 in barley. Silencing BEC 1019 resulted in much less sporulation and fungal growth. Thus, BEC 1019, may function to suppress

defense and enhance virulence during the development of powdery mildew in barley. BEC1019 homologs were detected in 22 other diverse pathogenic fungi, including the human pathogen *Candida albicans*, suggesting that BEC1019 may represent a large family of conserved fungal virulence factors.

PS07-306

Characterization of the *CoPRF1* mutant of *Colletotrichum orbiculare* defective in establishment of host infection

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Plant pathogens have co-evolved with their host plants which have evolved the defense system against their pathogens. It is generally accepted that plants express basal immunity by the recognition of the pathogen-associated molecular patterns, but compatible pathogens suppress the plant basal defense by secreting the effector protein. In our previous study, we have obtained several pathogenicity deficient insertional mutants in *Colletotrichum orbiculare* by *Agrobacterium tumefaciens*-mediated transformation (AtMT). Among them, in the mutant named YK4524 it was shown that a T-DNA insertion disrupted a gene which presumably encodes an extracellular protein with signal peptide sequence. And BLAST search of the predicted sequence found no significant homologous genes in published databases, suggesting that it is unique to *C.orbiculare*. So we named this gene *CoPRF1* (Pathogenesis-related factor1). Target gene disruption mutants of *CoPRF1* obtained by AtMT showed significant reduction in virulence on the host leaves. However, characteristics such as germination, appressorium formation and penetration hyphae formation of *coprf1* mutants *in vitro* were normal, indicating that *CoPRF1* is not essential for infection related morphogenesis. On the other hand, penetration ability of mutants was attenuated on intact cucumber cotyledons, and the elongation of its invasive hyphae was slower compared with the wild type. From these results, it was suggested that *CoPRF1* would engage in the establishment of host infection of *C.orbiculare*.

PS07-307

Functional characterization of secreted effector proteins from the hemibiotrophic fungal pathogen *Colletotrichum higginsianum*

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The ascomycete fungus, *Colletotrichumj higginsianum*, belongs to one of the most economically important genera of pathogens, causing anthracnose disease on a wide range of cruciferous plants, including *Brassica, Raphanus* and the model plant *Arabidopsis thaliana*. To identify fungal genes related to pathogenicity, we generated cDNA libraries from different *Colletotrichum* infection stages on *Arabidopsis* leaves, namely appressorial penetration, biotrophic phase and necrotrophic phase, and those libraries were deeply sequenced by 454-pyrosequencing. Secreted effector proteins enable plant pathogenic fungi to manipulate host defense responses for successful infection. By computational mining of the ESTs, we have identified sets of genes encoding putative *C. higginsianum* effector candidate (ChECs) that are specifically expressed in particular fungal infection structures. We identified two secreted LysM domain proteins from a biotrophic hypha-EST library, which may function as effectors to evade chitin-triggered immune responses. We are also evaluating the biological activities of ChECs in planta by *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana*. Several putative functions will be presented.

PS07-308

Identification of novel bacterial effector protein involved in hypersensitive response (HR) cell death in rice

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Plant pathogenic bacteria *Acidovorax avenae* N1141 rice-avirulent strain induces the plant immune responses containing hypersensitive (HR) cell death in rice. It has known that plant HR cell death was induced by several effector proteins secreted into plant cells through the bacterial Type III secretion system (T3SS). To clarify the induction mechanism of HR cell death in rice by N1141 strain, we analyzed genome sequence of N1141 strain and found 30 kbp *hrp* gene cluster encoding T3SS. The deletion mutant of T3SS in N1141 strain (*NΔT3SS*) did not induce HR cell death in rice, showing that effectors secreted through T3SS is involved in induction of HR cell death in rice. Therefore we next attempted to identify these effectors using proteome analysis. Several proteins of *NΔT3SS* strain were specifically accumulated compared with N1141 strains after inoculation to culture rice cells. These accumulated proteins were identified and disruption mutants of each gene encoding the identified protein were prepared. Among these disruption mutants, *Ahp1* disruption mutant did not causes HR cell death. Transient expression of *Ahp1* in rice cells caused HR cell death associated with nuclear DNA fragmentation, Furthermore, Ahp1 proteins were secreted into hrp minimal medium from N1141 strain, suggesting that Ahp1 is the effector involved in induction of HR cell death.

PS07-309

A homologue of an avirulence gene in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* race 1 functions as a virulence gene in the cabbage yellows fungus *F. oxysporum* f. sp. *conglutinans*

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Six4 is a small protein secreted by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in tomato xylem sap during infection (Houterman 2007). This protein triggers FOL race 1-specific resistance (*I*) of tomato, and *SIX4* is regarded as an avirulence gene (Houterman 2008). Although *SIX4* has been considered to be unique in FOL race 1 (Lievens 2009), we found that *F. oxysporum* f. sp. *conglutinans* (FOC) possesses a *SIX4* homologue in its genome (Kashiwa 2010). In this study, we analyzed the structure and function of the *SIX4* homologue in FOC. The *SIX4* in FOL and the *SIX4* homologue in FOC were 99% identical in nucleotide level (Kashiwa 2012). In a FOC isolate Cong: 1-1, *SIX4* located on a ca. 2 Mb small chromosome. The expression of *SIX4* was detected in the tissues, such as stems and roots, of cabbage infected with FOC Cong: 1-1 by RT-PCR. *SIX4* homologue-disruptants in FOC Cong: 1-1 did not gain virulence to FOC-resistant cabbage cvs. Syutoku-SP and Koikaze. On the other hand, the disruptants showed the reduction of virulence against FOC-susceptible cv. Shikidori. These suggested that the *SIX4* homologue is involved in virulence, but not in avirulence in FOC.

PS07-310

Elucidation of activation mechanisms of R protein Pit by the effector protein Avr-Pit

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Plants have immune systems against pathogens such as blast fungus. Pathogens secrete their effectors into plant cells. Plants perceive signals from invasion of effectors through disease resistance (R) proteins. Recognition of effectors by R proteins triggers rapid and effective defense responses called hypersensitive response. Most R proteins belong to the NB-LRR family proteins as they contain a central nucleotide-binding domain (NB) and C-terminal leucine-rich repeat (LRR) domain. We have recently found that small GTPase OsRac1 interacts with a NB-LRR-type R protein Pit and contributes to Pit-mediated defense response against rice blast fungus Magnaporthe grisea. Thus, OsRac1 acts as a direct signaling partner downstream of Pit. However, an activation mechanism of Pit is largely unknown because Avr-Pit has not been identified until now. To isolate Avr-Pit, we introduced a method based on whole-genome resequencing of pooled DNA from a segregating population of blast fungi that show Avr-Pit phenotype. Blast fungi carrying Avr-Pit were crossed directly to those without Avr-Pit, allowing unequivocal segregation in first filial generation (F(1)) lines of subtle phenotypic differences. We applied this method to two sets of blast fungus and identified several candidates of Avr-Pit. To further screen Avr-Pit, we will perform cell death assay using rice protoplasts to monitor the interaction between Avr-Pit and Pit.

PS07-311

Phosphatidylinositol monophosphate-binding ability of *Phytophthora infestans* RXLR effector AVR3a is required for the virulence function

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Pathogens deliver a number of effector proteins into plant cells to suppress PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI). Resistant plants are able to recognize the effectors by the resistance (R) proteins and induce strong immune responses. AVR3a, an effector protein secreted from potato blight pathogen *Phytophthora infestans*, has an RXLR motif at the N-terminus and is translocated into plant cells in the RXLR motif dependent manner. AVR3a suppresses PTI induced by the recognition of INF1. However, its underlying mechanism is still unclear. The NMR analysis revealed that the effector domain of AVR3a comprises four α-helices and has a positively charged surface area, which is important for binding phosphatidylinositol monophosphates (PIPs). AVR3a with a point mutation in the area was not able to suppress INF1-induced PTI, although it was still recognized by R3a, a potato R protein. Likewise, the stability of CMPG1 which is a virulence target of AVR3a was diminished by the mutation. In fact, the steady-state levels of the non-PIP-binding mutant proteins were significantly reduced. Furthermore, overexpression of PIP 5-kinase which phosphorylates PIPs resulted in the reduction of AVR3a protein levels *in planta*. These data suggest that the PIP-binding ability of AVR3a is essential for its accumulation *in planta* to suppress CMPG1-mediated immunity. We will discuss the molecular relationship between the lipid binding, the virulence function and the RXLR motif.

PS07-312

OsBPC1 targeted by Xoo1488 effector regulates chitin induced immunity in rice

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Plant bacterial pathogens equipped with the type III secretion system (TTSS) and generally deliver different TTSS effector proteins into plant cells. These TTSS effector proteins modulate the function of crucial host regulatory molecules and allow bacteria to invade plant cells. So far, we found that the transgenic rice plants expressing Xoo1488, one of the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) effectors showed severe susceptibility to the TTSS-deficient *hrpX* mutant of *Xoo*. Over-expression of Xoo1488 also suppressed chitin-induced immune response in rice suspension cell. We identified BASIC PENTACYSTEINE1 (OsBPC1) as potential interacting proteins of Xoo1488 using yeast two-hybrid screening. OsBPC1 is a plant specific transcription factor and localized in nucleus in rice. BiFC experiments indicated that Xoo1488 interacted with OsBPC1 in perinuclear region, suggesting that Xoo1488 may inhibit nuclear localization of OsBPC1. Electromobility shift assays revealed that OsBPC1 is able to bind GAGA element. Microarray analysis using transgenic rice suspension cell over-expressing *OsBPC1* showed that OsBPC1 regulates expression of chitin induced genes which have GAGA elements in their promoter regions. Furthermore over-expression of *OsBPC1* enhanced resistance to blast fungus (*Magnaporthe oryza*) in rice, suggesting that OsBPC1 plays important role in rice immunity.

PS07-313

***Magnaporthe oryzae* AVR-Pia protein: induction of resistance reaction in *Pia* rice and preparation of anti-AVR-Pia antibody**

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The avirulence gene *AVR-Pia*, which induces hypersensitive reaction (HR) of rice cultivars with the resistance gene *Pia* was isolated from *Magnaporthe oryzae* strain Ina168 (Miki *et al*., 2009) and gene expression during infection was associated by qRT-PCR using mRNA extracted from blast inoculated rice leaf, detected in 24 hours after inoculation. Furthermore, AVR-Pia protein localization to biotrophic interfacial complex (BIC: Khang *et al*., 2010) was observed using AVR-Pia::eGFP fusion protein in compatible rice leaf sheath cells. On the other hand, the functional detail of AVR-Pia protein during infection was not understood except that AVR-Pia interacts with other AVR-Pia molecule, which was suggested by yeast two-hybrid assay. In order to analyze the function of AVR-Pia protein, the recombinant AVR-Pia protein was purified from *E. coli*. Recombinant AVR-Pia solution was revealed to induce HR-like browning spots when it was infiltrated into *Pia* rice leaf, suggesting that the recombinant AVR-Pia has activity to trigger the host's resistance reaction. An anti-AVR-Pia antibody was prepared with the recombinant protein, and its validity was investigated by Western blotting. Native AVR-Pia was detected from total protein extracted from rice leaf sheath cells, and the MW of AVR-Pia was estimated as 7.4 kDa, corresponding to AVR-Pia w/o signal peptide. These results suggest that recombinant AVR-Pia has similar structure to the native AVR-Pia, and the antibody has specificity to native AVR-Pia and is useful for AVR-Pia localization after secretion during blast infection by immuno-staining.

PS07-314

New clues to the functions of AWR effectors from *Ralstonia solanacearum* by heterologous expression in yeast

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The present work focuses on the characterization of a multigenic family of 5 type III effectors called AWR from the plant pathogen *Ralstonia solanacearum*. These effectors are involved in bacterial infection as previous experiments *in planta* showed that their deletion renders the bacterium less virulent on tomato (Sole et al., 2012). In order to discover new clues to the functions of AWR effectors, we examined the consequences of induced expression of these genes (using Gateway-compatible yeast vectors) on yeast growth. Expression of T3SS effectors in *Saccharomyces cerevisiae* oversteps the limitations of their study in plants, as yeast lacks resistance (R) proteins that can trigger ETI responses. Production of AWRs from a galactose-inducible GAL1 promoter determined their classification into 3 groups according to their effect on yeast growth: AWR5 and AWR2 gave rise to the strongest toxicity, while AWR1 and AWR3 only inhibited yeast growth when their expression was induced. AWR4 had no effect on yeast growth. These results were confirmed by expression of *awrs* using a tetracycline-responsible (Tet-Off) promoter system both from episomic and genome-integrated constructs. The high toxicity of AWR5 was analyzed by growth curves of yeast transformed cells using the Tet-Off system and real-time RT-PCR. The nature of the growth restriction caused by AWR effectors is currently under scrutiny. Data will be presented on the involvement of the main physiological processes (e.g. respiration, membrane integrity) and the nature of this phenotype (growth arrest or cell death). Our results confirm yeast as a model system to identify gain-of-function phenotypes.

PS07-315

Using heterologous expression approaches to study the biological functions of *Xanthomonas campestris* pv. *campestris* type III effectors (T3Es)

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The plant-pathogenic xanthomonads modulate plant gene expression and overcome plant defenses by translocating a subset of type III effector proteins (T3Es) into plant cells via the type III secretion system (T3SS). In this study, we characterize the biological functions of *X. campestris* pv. *campestris* (Xcc) T3Es by heterologous expression approaches using *X. campestris* pv. *raphani* (Xcr) as a recipient bacterium and *Agrobacterium tumefaciens* for in-planta transient expression. Xcc causes systemic black rot disease on Brassicaceae, whereas Xcr elicits localized necrotic spots on the leaves of Brassicaceae and Solanaceae. Comparison of genome sequences reveals that the two xanthomonads harbor different repertories of T3Es, which are likely to be involved in their differential interactions with plant cells. Four of the differential Xcc *t3es*, *avrXccC*, *xopD*, *xopN*, and *xopX*, were cloned and ecotopically expressed in Xcr to test if the Xcc T3Es alter the interactions of Xcr with plant cells by monitoring bacterial growth and symptom development in cabbage leaves. In addition, the four effectors were transiently expressed in tobacco (*Nicotiana benthamiana* and *N. tabacum*) and tomato (*Solanum esculentum*) via *Agrobacterium*-mediated transformation, followed by a challenge-inoculation with wild-type Xcr, to assay for the Xcc T3SEs functions in planta. The results of *Agrobacterium*-mediated transient assay showed that XopX can induce HR in tobacco and tomato, whereas XopN promotes symptom development in tobacco upon Xcr inoculation. Our results indicate the heterologous expression method could be a feasible approach to analyze the functions of T3SEs for facilitating bacterial multiplication and eliciting symptoms in plants.

PS07-316

Effectoromics of the phytopathogenic nematode, *Globodera rostochiensis*

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The potato cyst nematode *Globodera rostochiensis* is presumed to employ multiple secreted effector proteins to successfully infect their host plants. Although several candidate effectors have been identified, their functions are as yet poorly defined. We computationally identified forty predicted secreted proteins of *G. rostochiensis* from NCBI and expressed sequence tag (EST) databases. We have generated a library of 38 clones corresponding to 28 genes, encoding predicted secreted proteins, in three different constructs for transient and systemic *in planta* expression. In an effort to elcucidate the biological functions of these predicted secreted proteins, we expressed them in *Nicotiana benthamiana*, *N. tabacum*, *Solanum tuberosum* and *S. lycopersicum*. Several of these effectors produce different phenotypes when transiently and systemically expressed *in planta*. Two of the effectors GrSPRYSEC-15 and GrEXPB2 induced cell death responses in *N. tabacum* and *S. lycopersicum* respectively, while their expression in *N. benthamiana* induced severe symptoms, including chlorosis and dwarfing. In addition, more than 40 % of the putative effectors produced dramatic phenotypes when expressed systemically in *N. benthamiana* by *Potato virus X* (PVX) based constructs. Furthermore, we have found that several effectors appear to render recombinant PVX avirulent on certain genotypes suggesting that these may be avirulence proteins recognized by plant resistant proteins. We have also found that several effectors can suppress cell death induced by cell death inducers suggesting that they may suppress plant defense responses. The phenotypes induced by these effectors and their possible biological roles in suppressing host immunity and establishing successful infection will be discussed.

PS07-317

Effector protein trafficking from *Piriformospora indica* and their function in barley root cells

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One of the exciting developments in plant-microbe interactions has been the finding that both pathogenic and mutualistic fungi deliver effector proteins into the cytoplasm of host cells. Deployment of a large set of effectors, which function either outside (apoplastic) or inside (cytoplasmic) the host cell, is postulated to be essential for successful colonisation of plant tissue. We are investigating the novel sebacinalean symbiosis zooming in on the model interaction between barley (*Hordeum vulgare*) roots and the basidiomycete *Piriformospora indica*, a mutualistic endophyte that alleviates salt stress and induces systemic resistance to fungal and bacterial diseases. Here, we address the secretion and translocation into host cells of *P. indica*'s effector proteins . One expanded family of *P. indica*'s putative effectors, named DELDs, is defined by a conserved pattern of seven amino acids (RISDELD) at the C-terminus. We are using mutational analysis in *P. indica* combined with heterologous expression of DELD proteins in barley roots to investigate the molecular role of DELD proteins in the mutualistic interaction between *P. indica* and plants.

PS07-318

The *Pseudomonas syringae* HopA1 effector is differentially recognized by plants and resembles phosphothreonine lyases from animal pathogens

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Pseudomonas syringae is a host specific plant bacterial pathogen that requires a type III protein secretion system to inject effector proteins into plant cells for pathogenicity. The type III effector HopA1 (formerly HopPsyA) was first characterized in *P. syringae* pv. *syringae* 61 and is encoded by a gene located in the DNA cluster that encodes the type III apparatus. *P. syringae* pv. *tomato* DC3000 contains a *hopA1* allele in a different region of the chromosome. $HopA1_{Psy61}$ and $HopA1_{PtoDC3000}$ proteins are 57% identical. In *Nicotiana tabacum* cv. Xanthi (tobacco) and *Arabidopsis thaliana* accession Ws-0, $HopA1_{Psy61}$ but not $HopA1_{PtoDC3000}$ elicits a hypersensitive response (HR), consistent with $HopA1_{Psy61}$ being recognized by a plant immune receptor inducing effector-triggered immunity. The C-terminal two-third of $HopA1_{Psy61}$ is recognized by tobacco whereas the N-terminal third is recognized by Arabidopsis suggesting that this protein is recognized differently by these plant species. Expression of HopA1 in yeast revealed that $HopA1_{Psy61}$ but not $HopA1_{PtoDC3000}$ inhibits yeast growth, which may suggest these effectors have different virulence targets. HopA1 shares sequence similarity with the *Photorhabdus luminescens* insecticidal toxin Mcf2 and the $HopA1_{PtoDC3000}$ structure, covering residues 122-380, resembles phosphothreonine lyases from animal pathogens, including the *Shigella* OspF and the *Salmonella* SpvC proteins. Five conserved residues between HopA1 and Mcf2, which also correspond to the residues predicted to be in the phosphothreonine lyase active site, were mutated to alanine. The majority of these residues were important for the HopA1-dependent phenotypes consistent with HopA1 being an enzyme structurally related to phosphothreonine lyases.

PS07-319

Characterization of *CbAve1* from *Cercospora beticola*

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The hemibiotrophic fungal pathogen *Cercospora beticola* causes leaf spot of sugarbeet. Despite perennial wide-spread losses from leaf spot, extremely little is known about the effectors that help to establish disease. It was shown previously that transient co-expression of *C. beticola* effector gene *CbAve1* and the tomato resistance gene *Ve1* resulted in a hypersensitive response in *Nicotiana benthamiana*. To assess the role of *CbAve1* during colonization, *CbAve1* knock-out mutants were generated and will be compared to the progenitor isolate during colonization of sugarbeet. In addition, quantitative RT-PCR of *CbAve1* during a growth on sugarbeet will be presented.

PS07-320

Transcriptome profiling identifies a novel *Xanthomonas* TALE-specific plant resistance gene in pepper

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During infection gram-negative plant pathogenic bacteria of the genus *Xanthomonas* inject a cocktail of 20-30 effector proteins into plant host cells. Transcription Activator- Like Effector Proteins (TALEs) are a structurally distinct class of effectors that function as transcription factors within the plant cell. TALEs bind to and transcriptionally activate plant susceptibility (*S*) genes that promote disease. However, some TALEs also bind and transcriptionally activate plant resistance (*R*) genes thereby triggering a defense response that hinders the bacteria to multiply and spread in the host plant. The TALE protein AvrBs3 from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) transcriptionally induce the bell pepper (*Capsicum annuum*) resistance gene *Bs3* which results in *Xcv* resistance. The *Xcv* TALE protein AvrBs4 is 97 % identical to AvrBs3 but is not recognized in pepper *Bs3* plants. However AvrBs4 triggers a defense response in the *Capsicum pubescens* accession PI 235047 that carries the resistance gene *Bs4C*. Due to the fact that AvrBs4 C-terminal deletion derivatives lacking acidic activation domain are not longer able to induce a cell death response we assume that AvrBs4 also transcriptionally activates the expression of *Bs4C*. Based on this hypothesis we aimed to identify *Bs4C* via differential transcript profiling using next-generation sequencing (NGS) and compared transcript populations of the resistant (PI 235047) and the susceptible accession (PI 585270) upon inoculation with AvrBs4 containing *Xcv* strains. One prime candidate for the pepper *Bs4C* gene was identified and could be confirmed by genetic mapping and complementation assays. Further characterization of *Bs4C* will be presented.

PS07-321

Development of novel fluorescent tags to monitor bacterial effector delivery *in vivo*

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To subvert the plant immune system and establish disease, gram-negative pathogens utilize the Type 3 Secretion System (T3SS) to deliver effectors into the host cell cytoplasm. Effector- or T3SS-knockouts are hypo- or non- virulent, demonstrating that successful delivery is crucial for efficient host colonization.Direct visualization of effectors while infections unfold *in situ* could provide many new insights into the mechanistics of infection, especially regarding dynamic aspects of these processes (e.g. timing and order of effector delivery, their subcellular targeting and turnover rates). Unfortunately, the fact that the well-established fluorophores (GFP / -derivates, RFP) block delivery of effectors by the T3SS has prohibited this type of studies to date. Instead, researchers have to resort to biochemical assays, which require isolation of proteins prior to analysis and consequently cannot provide subcellular resolution and real time monitoring, or to ectopic expression of effectors inside of host cells, which complicates the interpretation of results due to unrealistically high protein abundance.We are currently testing different strategies that could allow real-time tracking of bacterial effectors during their delivery *in vivo*. One approach, using the novel fluorophore iLOV, has been complicated by the fact that, despite a fold completely different from GFP, iLOV also interferes with type 3 secretion. However, an alternative system based on an unusual split-GFP system that has recently been demonstrated to work in Salmonella and tissue culture has so far passed all functional tests, making it a promising candidate. We will present our latest results on both these approaches.

PS07-322

Computational and molecular identification of *Xanthomonas oryzae* TAL effector targets in rice

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Transcription activator-like (TAL) effectors found in *Xanthomonas* species promote bacterial growth, disease susceptibility and defense responses by directly binding to *cis*-regulatory DNA

sequences and inducing host gene expression. In this study, we have compared the gene expression changes of rice (*Oryza sativa*) infected with the vascular and the non-vascular bacterial pathogens, *Xanthomonas oryza*e pv. oryzae (Xoo) and *Xanthomonas oryzae* pv. oryzicola (Xoc), respectively. Xoc and Xoo execute largely different transcriptional profiles and the most significant gene expression changes depend on a functional type three-secretion system (T3SS). To investigate the role of TAL effectors in the host gene expression, we used a computer-based matrix to predict host induced-genes that are targets by TAL effectors of Xoc and Xoo. Although meaningful TAL effectors were characterized in Xoo, their functions in Xoc remain unknown. Therefore we focused the study on Xoc TAL effectors. In total, we confirmed fifteen host genes that are directly targeted by seven different TAL effectors. Next we conducted in planta virulence experiments to associate the contribution of these TAL effectors to virulence. A TAL effector mutant that is complemented by Tal2g, appeared as the most reduced in virulence. We previously found that Tal2g induces two most significantly Xoc-induced genes. To discover the important target for Tal2g virulence, we designed customized TAL effectors targeting each specific Tal2g targets. Finally, we present the principal host susceptibility candidate for rice disease caused by Xoc.

PS07-323

Three new pathogenicity effectors of Pierces disease in *Xylella fastidiosa* not found in biocontrol strain EB92-1

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Xylella fastidiosa (Xf) infects a wide range of plant hosts and causes economically serious diseases, including Pierces Disease (PD) of grapevines. Xf biocontrol strain EB92-1 is infectious to grapevines but does not cause symptoms. The draft genome of EB92-1 revealed: 1) that it was nearly identical in gene order and sequence to Temecula; 2) no unique or additional genes were found in EB92-1 that were not previously identified in Temecula, and 3) EB92-1 appeared to be missing genes encoding 10 potential pathogenicity effectors found in Temecula (Zhang et al 2011). The latter included a type II secreted lipase (LipA; PD1703), two identical genes encoding proteins similar to zonula occludens toxin (Zot; PD0915 and PD0928) and all six predicted hemagglutinin-like proteins (PD0986, PD1792, PD2108, PD2110, PD2116 and PD2118). PCR analyses and subsequent sequencing of the PCR products confirmed that all 10 genes were missing in EB92-1. Leaves of tobacco and citrus inoculated with crude protein extracts of the Temecula PD1703 gene over-expressed in *E. coli* exhibited hypersensitive cell collapse in less than 24 hrs. PD1703, driven by its native promoter, conferred strong secreted lipase activity to *Xanthomonas citri, E. coli* and EB92-1 in plate assays. Pathogenicity of the EB92-1 exconjugant with PD1703 showed significantly increased symptoms on grapes as compared with an EB92-1 exconjugant carrying the empty vector. Similarly, Temecula PD0928 (Zot) and PD0986 (hemagglutinin) were also moved into EB92-1, both exconjugants also showed significantly increased symptoms on grape in comparison to EB92-1 with the empty vector.

PS07-324

GRP7, a substrate of *Pseudomonas syringae* type III effector HopU1, plays a role in plant innate immunity by binding to immunity-related RNA

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The bacterial pathogen *Pseudomonas syringae* uses a type III secretion system (T3SS) to inject effectors (T3Es) into plant cells and suppress plant immunity. The *P. syringae* pv. *tomato* DC3000 T3E HopU1 was determined to be a mono-ADP-ribosyltransferase that can use several RNA-binding proteins as substrates that have RNA-recognition motifs (RRMs). One of these proteins, GRP7 was shown to be involved in innate immunity and Arabidopsis mutants lacking GRP7 were more susceptible to *P. syringae*. HopU1 ADP-ribosylates an arginine residue in position 49 of GRP7, which is within its RRM. We found that ADP-ribosylated GRP7 was reduced in its ability to bind RNA. Also, transgenic grp7 mutant plants expressing GRP7 restored the susceptibility and immunity-related phenotypes associated with the mutant plant to wild type plant phenotypes whereas transgenic plants expressing a GRP7R49K protein did not indicating that the amino acid that is the site of ADP-ribosylation is critical for GRP7's function. Recently, we found that plants over-expressing GRP7 were more resistant to *P. syringae* and other pathogens supporting that GRP7 plays a broadly important role in innate immunity. Yeast two hybrid screens and in planta co-immunoprecipitation experiments indicate that GRP7 interacts with several proteins involved in translation. To screen RNAs modulated by GRP7, we performed RNA-immunoprecipitation followed by RNA sequencing (RIP-Seq). Thus far, our RIP-Seq results indicate that GRP7 binds to several immunity-related RNAs. Taken together, GRP7 likely interacts with several immunity-related RNAs to insure that these RNAs are efficiently translated such that the plant can mount a robust immune response.

PS07-325

The *Pseudomonas syringae* type III effectors HopK1 and AvrRps4 are processed during import into chloroplasts

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To be pathogenic *Pseudomonas syringae* injects effector proteins into plant cells via its type III secretion system. A primary role for the majority of these effectors is to suppress plant immunity. We focused on one of these effectors, HopK1, because it possessed a robust ability to suppress immunity. A *P. syringae* pv. *tomato* DC3000 *hopK1* mutant was substantially reduced in virulence more so than most single effector mutants, which usually have subtle virulence phenotypes. The N-terminal 147 amino acids of HopK1 share high sequence identity with the well characterized AvrRps4 protein, however, their C-terminal regions are dissimilar. HopK1 is processed inside plant cells at the same site where AvrRps4 has been reported to be processed. Interestingly, transgenic plants expressing HopK1 and AvrRps4 derivatives indicate that both proteins are targeted to chloroplasts using subcellular localization and biochemical fractionation. Additionally, biochemical fractionation experiments using Arabidopsis infiltrated with *P. syringae* strains containing HopK1-HA or AvrRps4-HA indicate that the processed form of these bacterially-injected proteins was found predominately in chloroplasts. Immunity-induced transgenic plants expressing full length HopK1 were reduced in two common immune responses and complemented the virulence phenotype of a DC3000 *hopK1* mutant. However, plants expressing the processed form of HopK1, which would not be localized to chloroplasts, failed to complement the DC3000 *hopK1* mutant and produced immune responses similar to wild type plants suggesting the HopK1 needs to be localized to the chloroplast to function. Taken together, HopK1 and AvrRps4 likely target distinct plant proteins inside chloroplasts to contribute to plant pathogenesis.

PS07-326

Xanthomonas Type III effector XopD desumoylates tomato transcription factor SlERF4 to suppress ethylene responses and promote pathogen growth

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Manipulation of host protein sumoylation by pathogens has emerged as an important virulence strategy to suppress immunity. The direct link between protein sumoylation and eukaryotic transcription suggests that pathogens might directly modulate the sumoylation state of transcription factors. Here we provide evidence that XopD, a SUMO protease from *Xanthomonas campestris* pathovar *vesicatoria* (Xcv), directly interferes with plant transcription to modulate ethylene (ET) responses during infection. XopD is required to promote Xcv growth in tomato leaves and to suppress disease symptom development. Given that XopD contains two EAR motifs implicated in ET signaling and transcription repression, we hypothesized that XopD may directly regulate ET production and/or signaling. Consistent with this hypothesis, ET gas and biosynthesis mRNAs were significantly higher in Xcv delta*xopD*-infected leaves compared to Xcv-infected leaves. Both ET production and perception were required for tomato immunity and symptom development. Inspection of tomato ERFs expressed in Xcv-infected leaves suggested that SlERF4 is a putative XopD substrate. Virus-induced gene silencing in tomato revealed that *SlERF4* mRNA expression was required for Xcv delta*xopD*-induced ET production and ET-stimulated immunity. XopD was found to colocalize with SlERF4 in subnuclear foci and hydrolyze tomato SUMO1 from K53 of SlERF4 resulting in SlERF4 destabilization. Mutation of K53 to R53 prevented SlERF4 sumoylation, decreased SlERF4 levels, and reduced SlERF4-dependent transcription. We conclude that XopD directly binds and desumoylates SlERF4 to repress ET induced-transcription required for Xcv immunity. This is the first example of a pathogen SUMO protease that targets a host sumoylated transcription factor to suppress defense.

PS07-327

Dissecting the interaction between *P. syringae* pv. *phaseolicola* and its non-host *A. thaliana* using effectoromics

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MAMP-triggered immunity (MTI) is thought to be a major determinant of non-host resistance (NHR) of Arabidopsis to *P. syringae* pv. *phaseolicola* 1448A (Pph1448A). However, Pph1448A produces effectors that can be potentially recognized and induce effector-triggered immunity (ETI) in Arabidopsis including AvrRps4, HopAS1, and three AvrB homologs: AvrB2, AvrB4-1 and AvrB4-2. To establish the contribution of ETI to the incompatibility between Arabidopsis and Pph1448A, we determined the patterns of effector recognition among different Arabidopsis ecotypes. We used a Tobacco Rattle Virus-based transient expression system to deliver effectors individually and analyzed their ability to induce ETI based on the phenotype of infected plants. Recognition was manifested as symptomless immunity or as extensive necrosis associated with the induction of a hypersensitive response. All three AvrB homologs triggered RPM1/RIN4 and TAO1-mediated defenses in Col-0. In addition, two AvrB4 paralogs triggered RPM1/TAO1-independent defenses in Col-0 due to RPS2 activation. Also HopJ1 triggered defense responses in Col-0 and several other ecotypes. We mapped this response to a ~10cM region in the Arabidopsis genome and using a reverse genetic approach narrowed down the determinant of recognition to a single CC-NB-LRR-encoding gene with no known specificity reported previously. We named this gene *DERK1* for *Determinant of Effector Recognition 1*. We are pyramiding several knockouts of NB-LRR encoding genes to produce lines that are compromised in recognition of effectors from Pph1448A. These will enable us to determine the quantitative contributions of ETI and MTI to NHR of Arabidopsis to Pph1448A.

PS07-328

Identification and characterization of intracellular effectors Crinklers of the Oomycete *Aphanomyces euteiches*, a root pathogen of legumes

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Aphanomyces euteiches is an oomycete infecting roots of various legumes species as pea, alfalfa and the model legume *Medicago truncatula*. The genus *Aphanomyces* (Saprolegniales) has a particular taxonomic position within oomycetes comprising both animal pathogen and plant pathogen species. cDNA libraries from infectious mycelium revealed the presence of ortholog CRN (Crinkling and Necrosis) genes, initially identified in *Phytophthora infestans*. CRN proteins of *Phythophthora sp* are coded by several hundreds of genes and have been classified in different families according to sequence features on their carboxyl terminal domains. While these Cterminal domains are variable and are thought to be implicated in the function of the protein, the Nterminal domains are highly conserved and characterized by the presence of a LFLAK amino acid motif implicated in the translocation from the pathogen to the host cell. *A.euteiches* expresses during infection two families of CRNs, AeCRN5 and AeCRN13, both presenting a LYLALK motif responsible for the internalization of the protein inside plant cells. Both proteins are expressed during infection of *M. truncatula* roots. *In planta* expression of both proteins has revealed that AeCRN5 and AeCRN13 are targeted to the nucleus. Their expression in roots alters root architecture by inhibiting root development, while triggerring cell death in *N. benthamiana* leaves. Such observations suggest that *A. euteiches* 's CRNs are virulence proteins exerting their function through the interaction with nuclear compounds. Latest results concerning their characterization will be presented in the poster.

PS08-329

Seeing the world outside: a virus uses the host sensorial system to take cues from the environment

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Viruses rely totally on the host to achieve every step of the infection cycle. Much is known about how viruses interfere with cellular processes to put them at their use and it is clear that they intercept intracellular and intra-host communication and processes to optimise interaction with the host. Here we unprecedentedly that show viruses are also able to use the host sensorial system to very rapidly perceive and react on cues from the world outside the host, in a way disconnected from the reaction of the host itself. *Cauliflower mosaic virus* (CaMV) is transmitted from plant-to-plant by aphids, and previous work has shown that the virus-aphid interaction is not an accidental process but depends on the presence of the virus-induced Transmission Bodies (TBs) in infected cells, containing the CaMV transmissible complexes. Our results demonstrate that TBs react on the presence and feeding of the insect vector by rapidly and reversibly dispersing their contents on cortical microtubules throughout the cell. If this TB reaction is perturbed, transmission rates drop; if this reaction is artificially enhanced, transmission rates rise. This shows that CaMV intercepts the host's perception of the aphid and immediately translates it in an appropriate response that optimises its chances of acquisition, everything going back to normal standby state a few minutes later.

PS08-330

Tomato SlSnRK1 protein interacts with and phosphorylates βC1, a pathogenesis protein encoded by a geminivirus betasatellite

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The βC1 protein of Tomato yellow leaf curl China betasatellite (TYLCCNB) functions as a pathogenicity determinant. To better understand the molecular basis of βC1 in pathogenicity, a yeast two-hybrid screen of a tomato cDNA library was carried out using βC1 as bait. βC1 interacted with a tomato SNF1-related kinase designated as SlSnRK1. Their interaction was confirmed using a bimolecular fluorescence complementation assay in *Nicotiana benthamiana* cells. Plants over-expressing SnRK1 were delayed for symptom appearance and contained lower levels of viral and satellite DNA, while plants silenced for SnRK1 expression developed symptoms earlier and accumulated higher levels of viral DNA. In vitro kinase assays showed that βC1 is phosphorylated by SlSnRK1 mainly on serine at position 33 (S-33) and threonine at position 78 (T-78). Plants infected with βC1 mutants containing phosphorylation-mimic aspartate residues in place of S-33 and/or T-78 displayed delayed and attenuated symptoms and accumulated lower levels of viral DNA, while plants infected with phosphorylation-negative alanine mutants contained higher levels of viral DNA. These results suggested that the SlSnRK1 protein attenuates geminivirus infection by interacting with and phosphorylating the βC1 protein.

PS08-331

Molecular characterization of *Chilli leaf curl virus* and satellite DNA associated with tomato in Oman

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Tomato is cultivated in the coastal region of the Sultanate of Oman during the winter season to meet the high demand for fresh produce in the domestic market. To identify the causal agent of a widespread disease associated with infestations of the whitefly, leaves were collected from tomato plants showing symptoms characteristic of the begomovirus disease in Al-Batinah and Dhofar regions during 2010 and 2011. Total nucleic acids were isolated from the tomato leaves and used as the template for rolling circle amplification of begomoviral DNA. The *Nco*I digested putative full length begomoviral DNA was cloned and sequenced. The complete nucleotide (nt) sequence was determined as 2758 bp, indicative of a monopartite begomoviral genome. The virus from Oman was most closely related to ChLCV-Multan at 92% nt identity. However, AV1 and AV2 ORFs of ChLCV-Om showed high nt similarity with PepLCV-Lahore and ChLCV-Panipat. Based on the guidelines of the ICTV the Oman isolate has been designated ChLCV-Om and is considered a strain of ChLCV-Multan. A satellite DNA was amplified by PCR using degenerate primers and cloned, and the DNA sequence was determined. Analysis of the complete nt sequence of 1327 bp indicated that the DNA β shared 96% similarity with its closest relatives, TYLCV Al-Batinah DNA-β isolated from tomato in Oman. This is the first report of ChLCV from Oman and DNA-β associated with the ChLCV-Om isolate. The ChLCV-Om and associated DNA-β thus represent a begomovirus-complex at the Asian-Middle East crossroads that uniquely share geographical and genetic hallmarks of both.

PS08-332

Functional analysis of *Cucumber mosaic virus* 2b protein and coat protein on symptom development of inoculated tobacco plant

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Cucumber mosaic virus (CMV) pepo strain caused leaf malformation with pale green chlorosis (mosaic symptom) on infected tobacco plant. Coat protein (CP) mutants of pepo, which P at residue 129 was substituted by C, Q or S, induced white chlorosis, while substitution by A, D or E, did not alter the pale green chlorosis. These CP mutants caused low expression of some photosynthesis-related genes that was correlated with few thylakoid membranes and chlorosis phenotype. Mutations of 2b protein, which R at residue 46 was substituted by C (R46C), or S at residues 40 and 42 were substituted by A (S40/42A), resulted in asymptomatic phenotype, regardless of the RNA silencing suppressor activity. Thus, CP and 2b are the virulence determinants of CMV in tobacco plant. To further understand the role of CP and 2b on symptom development, virulence of CMV mutants of combined substitutions with both of CP and 2b were analyzed. The 2b mutants (R46C or S40/42A) containing an amino acid substitution in the CP (129A, 129E, 129C, 129Q or 129S) induced chlorosis without leaf malformation, while the 2b mutants that an amino acid 129 in the CP was D or P showed asymptomatic phenotype. These results suggest that mutated pepo CP containing an amino acid 129A, 129E, 129C, 129Q or 129S solely triggers chlorosis while pepo CP with 129P or 129D does not have virulence. In addition, the wild type 2b protein of pepo probably determines leaf malformation with pale green chlorosis.

PS08-333

5' untranslated region of tobamovirus RNA is involved in viral cell-to-cell movement

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To establish a systemic infection in plants, viruses invade neighboring cells via cell-to-cell movement through plasmodesmata until they reach phloem. Movement protein (MP) of tobamoviruses plays critical roles in transporting viral nucleic acid and enlarging the pore size of plasmodesmata. In addition to MP, 130K/180K replicase proteins of tobamoviruses are reported to be involved in viral cell-to-cell movement by the yet unknown mechanism. In this study, we explored additional viral factors involved in tobamovirus cell-to-cell movement. In an analysis using chimeric viruses consisting of *Paprika mild mottle virus* Japanese strain (PaMMV-J) and *Tomato mosaic virus* (ToMV), replacement of 5' untranslated region (5'-UTR) of PaMMV-J with that of ToMV resulted in the inhibition of viral movement in tomato plants without affecting viral RNA replication. To further determine nucleotide sequences causing PaMMV-J movement inhibition, we have constructed mutant viruses in which several parts of PaMMV-J 5'-UTR were replaced with the corresponding nucleotides of ToMV. Nucleotide replacement in two distinct parts of PaMMV-J 5'-UTR, by which AUUAC pentanucleotide sequence was generated, resulted in the inhibition of the viral movement in tomato plants. Interestingly, this inhibitory effect appeared to be related to the origin of 130K/180K proteins: movement inhibition was not observed in the chimeric virus with 130K/180K replicase proteins from ToMV. These observations suggested that 130K/180K replicase proteins of tobamoviruses are involved in the viral movement, possibly by interacting with nucleotide sequence in the 5'-UTR.

PS08-334

Characterization of a ribonucleoprotein complex that serves as a precursor of tobacco mosaic virus replication complex

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Tobacco mosaic virus (TMV) is a positive-strand RNA virus. After invasion of host cells, the genomic RNA of TMV is translated to yield a 130-kDa protein and its read-trough product of 180 kDa. The 130-kDa and 180-kDa proteins (hereafter, the "replication proteins") recruit the genomic RNA to the cytoplasmic surfaces of organellar membranes to form the replication complex. In the replication complex, negative-strand RNA is synthesized, and then using it as a template, a large amount of progeny RNA is produced. We have previously established an in vitro system in which TMV RNA is translated and replicated in a cell-free extract of evacuolated tobacco protoplasts (BYL). When TMV RNA was translated in BYL from which membranes had been removed by centrifugation, RNA replication did not occur but a ribonucleoprotein complex that contained TMV RNA and the replication proteins accumulated. This ribonucleoprotein complex could form active replication complex upon addition of BYL membranes. Here, we show that the replication proteins bind a specific region of TMV RNA in this ribonucleoprotein complex, and that TMV RNA in the complex is a less active template for translation than purified TMV RNA. The genomic RNAs of positive-strand RNA viruses should serve as templates for both translation and negative-strand RNA synthesis, in which ribosomes and RNA polymerases move in opposite directions, and their collision will be fatal for both processes. To avoid ribosome-polymerase collision, inhibition of TMV RNA translation in the ribonucleoprotein complex may be a prerequisite for RNA replication.

PS08-335

Next generation sequencing reveals chrysanthemum genes and small RNAs associated with *Chrysanthemum stunt viroid*

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The chrysanthemum is one of popular flowers worldwide and is also important for floriculture industry. *Chrysanthemum stunt viroid* (CSVd) is a main pathogen leading to dramatic economic losses of chrysanthemum production. In order to identify genes associated with CSVd infection, we carried out transcriptome analysis of CSVd infected chrysanthemum using high-throughput Roche GS FLX454 pyrosequencing method. A total of 99,750 reads were obtained, trimmed, and assembled into 11,600 expressed sequence tags (ESTs), which were further annotated by the blast2go program. Comparative analysis with other plant genomes revealed about 70% of chrysanthemum ESTs are conserved in other plant species. In addition, we found that 208 chrysanthemum ESTs were assigned to various transcription factor families. To get enriched functions of obtained ESTs, we performed gene ontology (GO) enrichment analysis implemented in the GOEAST program and identified enriched 276 GO terms. Of them, GO terms related to chloroplasts, mitochondria, plasmodesmata, stress responses, and metabolisms were highly enriched. In addition, we determined small non-coding RNAs in CSVd infected chrysanthemum using Illumina Solexa sequencing identifying a large number of small RNAs derived from CSVd. This study is the first report which presents a transcriptome analysis of CSVd infected chrysanthemum as well as small RNAs related to CSVd using next generation sequencing. The obtained ESTs and CSVd derived small RNAs provide useful information to study plant-viroid interaction.

PS08-336

A putative sodium-hydrogen antiporter helps *Bamboo mosaic virus* accumulation in *Nicotiana benthamiana*

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Bamboo mosaic virus (BaMV), a potexvirus, has an approximately 6.4 kb positive-strand RNA genome with a 5' cap and 3' poly-(A) tail. ORF1 of the virus encodes a replication protein, consisting of a mRNA capping domain, a helicase-like domain and a RNA-dependent RNA polymerase (RdRp). The RdRp domain was used as bait to screen a leaf cDNA library of *Nicotiana benthamiana* by yeast two-hybrid screening. A putative sodium-hydrogen antiporter (NbNHAP) was found to interact with the bait. To understand the effect of NbNHAP on BaMV accumulation, the NbNHAP-silenced *N. benthamiana* was transfected with a BaMV infectious clone and BaMV coat protein accumulation was determined later. The result showed that the coat protein accumulation decreased in the NbNHAP-silenced plants. In protoplast assays, overexpression of NbNHAP increased the accumulation levels of BaMV coat protein. Together, these results suggest that NbNHAP may provide a favorable environment for BaMV replication.

PS08-337

Do non-circulative plant viruses sense the arrival of the aphid vector?

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CaMV (a DNA virus) and TuMV (a RNA virus) use the non-circulative mode for transmission by aphids: virus particles bind to a receptor located at the tip of the aphid stylets (proboscis-like mouthparts) when the aphids insert the stylets into cells while feeding on infected plants. When aphids change the plant, the viruses are transported in the stylets to a new host and inoculated into it. Our published results show that CaMV forms an intracellular transmission body (TB), that is specialized for transmission and that transmission requires living cells. This indicates that transmission of CaMV is not by accidental contamination of the vector mouthparts but results from specific interactions between the virus and the vector during the acquisition process. Our unpublished results (see also communication by Aurelie Bak) show that the TB reacts specifically on the arrival of the aphid vector and disintegrates rapidly and reversibly, thereby distributing transmissible virus complexes on microtubules throughout the cell and greatly enhancing transmission. Two questions arise: 1) Is CaMV the only virus able to sense the vector's arrival and prepare accordingly for transmission? 2) How does the virus sense the vector? Here we show that also transmission of the unrelated TuMV requires living cells and does not result from accidental contamination. A pharmacological analysis shows that calcium signalling is a very early step common for aphid sensing by both viruses, whereas downstream reactions to aphids differ. Taken together, we propose that vector sensing by viruses might be a general phenomenon enabling efficient transmission.

PS08-338

The interaction proteome of the N NB-LRR immune receptor

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Plants use NB-LRR immune receptors to recognize specific pathogen effectors and trigger defense responses. Identifying members of NB-LRR immune receptor multi-protein complexes will improve our understanding of pathogen recognition, immune receptor activation, and defense signal induction. Because NB-LRRs have low endogenous expression levels and are recalcitrant to over-expression, isolating NB-LRR complexes has been a challenge. Recent improvements in affinity purification and mass spectrometry (AP-MS) have it made it feasible to enrich NB-LRRs complexes and identify low-abundance members. Here, we performed AP-MS on the TIR-NB-LRR immune receptor N from *Nicotiana sp.*, which confers resistance to *Tobacco mosaic virus* (TMV). We fused genomic N to a tandem affinity purification tag (N-TAP) containing c-myc epitopes. As a control to identify non-specific co-purifying proteins, we used β-glucuronidase-GFP-TAP. Following one-step immunoaffinity purification, we used label-free LC-MS/MS to identify several novel N multi-protein complex members. We will discuss the role(s) of these proteins in N-mediated defense against TMV.

PS08-339

Management of whitefly transmitted begomovirus associated with tomato in Oman

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Tomato yellow leaf curl virus (TYLCV) is a whitefly-transmitted begomovirus. During field survey in 2010-2011 disease incidence on tomato associated with begomovirus was found to be 10-100%. Disease symptoms, which include yellowing, leaf curling and severe plant stunting are reminiscent of begomovirus. Tomato seedlings covered with Agryl net in nursery and 6-7 weeks after transplant showed less than 5% viral symptoms and high tomato yield. In transmission studies, female whiteflies were found more efficient in transmitting virus and associated satellite DNA as compared to male whiteflies. Viral acquisition and transmission rates by whitefly were evaluated by symptoms development and confirmed by PCR. The minimum acquisition period was found to be 30 min and transmission period 15 min for successful disease development. Seventeen-tomato breeding lines introgressed with Ty genes resistant to begomovirus, were challenged with viruliferous whiteflies. Nine out of seventeen breeding lines showed no symptoms and were field resistant, whereas eight breeding lines showed moderate to high susceptibility to TYLCV. Amplification fragment length polymorphism (AFLP) revealed a high polymorphism among all breeding lines. Most of the resistant breeding lines clustered together but some with susceptible ones indicating that clustering is due to their genetic relatedness and not the resistance. This can contribute to make some decisions by breeders in relation to the choice of the appropriate parents and linkage for the resistance genes.

PS08-340

Regulation of the cell-to-cell movement of plant viruses by a Ser/Thr kinase-like protein

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Studying the interaction between hosts and the viruses offers a potential way to develop antivirus strategies. In this study, we focus on a *Nicotiana benthamiana* Ser/Thr kinase-like (NbSTK-like) protein which is involved in the cell-to-cell movement of *Bamboo mosaic virus* (BaMV). BaMV is a single-stranded, positive sense RNA virus (Lin et. al., 1992) which causes significant economical lost of bamboo in Taiwan. By using cDNA-AFLP, the NbSTK-like protein is found to be up-regulated in the BaMV-inoculated *N. benthamiana* (Cheng et. al., 2007). NbSTK-like contains the homologous domain of Ser/Thr kinase. Knocking down the expression of NbSTK-like reduced the accumulation of BaMV in the inoculated leaves but not in the protoplasts. The localization of NbSTK-like is mainly on the cell membrane. Active site mutation of NbSTK-like does not change its subcellular localization but significantly affect the BaMV accumulation. These data implicate that NbSTK-like facilitates the cell-to-cell movement of BaMV. Moreover, knocking down the expression of NbSTK-like also affects the accumulation of *Cucumber mosaic virus* (CMV), thus NbSTK-like may have broader effect on different viruses rather than specific to BaMV. However, the phosphorylation status of BaMV coat protein was unaffected in the NbSTK-like knockdown protoplasts. The substrate of NbSTK-like and the detail mechanism are under investigation.

PS08-341

Mutations in the 130K/180K replication protein genes of *Pepper mild mottle virus* that confer the ability to systemically infect tomato plants reduce its infectivity in original hosts

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Pepper mild mottle virus (PMMoV) and *Tobacco mild green mosaic virus* (TMGMV) cannot replicate within tomato protoplasts due to the inhibitory effect exerted by the tm-1 protein. A mutant of TMGMV Japanese strain (TMGMV-J) with the T894M- and F970Y-substitutions in the 130K/180K replication protein (130K/180K) can replicate within tomato protoplasts and infect tomato plants systemically. In this study, we analyzed the effect of the corresponding amino acid changes (the insertion of R between the 889th and 890th residues and the F970Y-substitution) in PMMoV Japanese strain (PMMoV-J) 130K/180K on infectivity in tomato plants. Though the mutant with the 889R insertion and the F976Y-substitution (PMMoV-889/976) and that with the F976Y-substitution alone (PMMoV-976) replicated in tomato protoplasts, they did not infect tomato plants. The affinity of the 130K protein of PMMoV-889/976 toward tm-1 protein was lower than that of the wild-type PMMoV 130K protein. We then selected a spontaneous PMMoV-889/976 mutant which could systemically infect tomato plants. The mutant contained additional D1091N-substitution in the 130K/180K. Furthermore, this PMMoV mutant (PM-889/976/1091) systemically infected tomato plants. These results suggest that the F976Y-substitution is critical for overcoming tm-1-mediated resistance, and the D1091N-substitution is critical for systemic infectivity of PMMoV in tomato plants. Interestingly, either the F976Y- or D1091N-substitutions led to lower replication activity and lower systemic infectivity of the mutants in original hosts, *Capsicum annuum* and *Nicotiana benthamiana*. Thus, the PMMoV mutations, which enabled the virus to infect tomato plants, reduced the replication ability of the virus in original hosts.

PS08-342

Recapitulation of ribosomal frameshifting of *Clover yellow vein virus* P3N-PIPO in a cell-free translation system

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The family *Potyviridae* is one of the most agriculturally important

virus groups. Potyviruses have a single-stranded, positive-sense RNA genome of approximately 10 kb in length. The viral genome contains a single long open reading frame (ORF) encoding a polyprotein which is cleaved into approximately 10 mature proteins. Recently, a short ORF, pipo, was discovered within the P3 cistron of potyviral polyprotein (Chung *et al.*, *PNAS* 105; 5897-5902, 2008). The PIPO ORF exists in the +2 reading frame relative to the polyprotein. PIPO is proposed to be expressed as a protein fused to the N-terminal part of P3 (P3N-PIPO). However, whether P3N-PIPO is expressed via transcriptional slippage or ribosomal frameshifting is unknown. *Clover yellow vein virus* (ClYVV) is a member of the potyvirus group. ClYVV also contains the PIPO ORF in the P3 cistron. P3N-PIPO of ClYVV is suggested to be associated with the viral pathogenicity in pea carrying the resistance gene, *cyv1*. In this study, we investigated whether ClYVV P3N-PIPO is synthesized by ribosomal frameshifting, by using a cell-free translation system. We found that P3N-PIPO is expressed by ribosomal frameshifting. Furthermore, the frameshifting efficiencies are different between two ClYVV isolates: the one that is virulent to the *cyv1*-carrying pea expressed more P3N-PIPO than the avirulent isolate.

PS08-343

Multiple suppressors of posttranscriptional gene silencing encoded by *Ageratum yellow vein virus*, a monopartite begomovirus

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Gene silencing is a natural defense response of plants against invading viruses. In counter-defense, viruses encode suppressors of gene silencing that allow them to effectively invade plants. Ageratum yellow vein disease (AYVD) is caused by the association of a Tomato leaf curl Java betasatellite [Indonesia: Indonesia 1:2003] (ToLCJB-[ID:ID:03]) with a begomovirus component. *Ageratum yellow vein virus*-Indonesia [Indonesia:Tomato] (AYVV-ID[ID:Tom]) alone could systemically infect the plants and induced upward leaf curl symptoms even in the absence of betasatellite. However ToLCJB-[ID:ID:03] was required, in addition to AYVV-ID[ID:Tom], for induction of severe downward leaf curl disease in *N. benthamiana* plants. We have identified the DNA encoded V2 and its betasatellite that the V2 and betaC1 genes are symptom determinants. We also found that the ToLCJB-[ID:ID:03], encoded betaC1 proteins as efficient silencing suppressors of posttranscriptional gene silencing (PTGS) by using *Agrobacterium* co-infiltration or heterologous PVX vector assays. However, the results also showed weak suppression of gene silencing activities for C2 and C4 induced by GFP mRNA associated with GFP was detected. Furthermore, confocal imaging analysis of ToLCJB-[ID:ID:03] betaC1 in the epidermal cells of *N. benthamiana* shows that this protein is accumulated towards the periphery of the cell and around the nucleus, however, V2 accumulated in the cell cytoplasm, C4 associated with plasma membrane and C2 exclusively targeted into nucleus. In this study, we identified as many as four distinct suppressors of RNA silencing encoded by AYVV-ID[ID:Tom] and its cognate betasatellite in the family *Geminiviridae*, counteracting innate antiviral response.

PS08-344

Toward molecular isolation of the *Pvr4* gene conferring resistance against *Pepper mottle virus* in *Capsicum annuum*

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The plant viruses of *Potyviridae* family including *Pepper mottle virus* (PepMoV) and *Potato virus Y* (PVY) are known as most destructive plant viruses with disease symptoms of mottling on pepper or tomato leaves. In pepper, *Capsicum annuum* CM334 containing *Pvr4* and *Capsicum chinense* accession PI159236 containing *Pvr7* are reported as resistant sources against PepMoV and PVY. The *Pvr4* is known as a single dominant resistance gene against potyvirus with a broad spectrum and is located in chromosome 10. *Pvr7* is also determined as a single dominant gene, which has been known to be tightly linked to *Pvr4* in PI159236. On the basis of these, we try to isolate *Pvr4* gene by performing genetic analysis of PepMoV resistance with BC1F3 100 individuals from CM334 (*Pvr4*) and ECW123R (*pvr4*). PCAPS marker linked at a distance of 7cM from *Pvr4* has been developed through comparative genomics between tomato and pepper CM334 genome. Positional cloning of *Pvr4* by marker developments and candidate gene approach using pepper draft genome sequence is under way. Progress of our work on map-based cloning of *Pvr4* will be presented as a poster.

PS08-345

Screening for virulence factors of *Gentian Kobu-sho-associated virus* involved in tumorous or hyperplastic disorders in gentian

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Japanese gentians are perennial plants cultivated for ornamental purposes. Kobu-sho is a syndrome that causes tumorous or hyperplastic disorders on stems, nodes and roots of gentian, but the most common and early symptom is stunt with shortened internodes. Kobu-sho was first reported in the mid-1980s, but the causal agent(s) remains unknown. We have identified a novel virus-like double stranded (ds) RNA of approximately 23 kb, which showed statistically significant relevance to Kobu-sho and designated it as *Gentian kobu-sho-associated virus* (GKaV). GKaV sequence has a single large ORF that encodes a potential polyprotein of about 7,400 amino acids. The amino acid sequence did not show significant similarity to any plant viral proteins. To search for the virulence factors involved in Kobu-sho development, we expressed partial fragments of the GKaV sequence using a transient assay system based on *Nicotiana benthamiana-Potato virus X* (PVX) vector system because infectious GKaV molecular clone is not available. We amplified 55 overlapping fragments of 810 bp from GKaV genome and expressed them using PVX vector. We found that the expression of two fragments (GK1 or GK32) induced symptoms distinct from control vector expressing GFP. PVX/GK1 induced stunting and systemic cell death. PVX/GK32 induced the leaves to be curled, the stems to be bent and the whole plants to be reptant. Furthermore, it induced an ectopic development of leaf-like tissue on the abaxial side of veins in normal leaves. The results suggest that these fragments encompass the candidates for different virulence factors of GKaV.

PS08-346

Evaluation of the durability of *N'* resistance gene to *Pepper mild mottle virus* using random mutagenesis of coat protein genes

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Although the use of resistant cultivars is one of the most ideal strategies in plant disease control, the resistance-breaking pathogen strains sometimes break out and cause severe losses in the fields. Gene manipulation and some other technologies would provide us with opportunities to use the broken resistance genes for controlling diseases in different crops. It would be useful to use tobamovirus resistance genes, *L* and *N'* from pepper and *Nicotiana sylvestris*, respectively, in combination to control tobamovirus infection in pepper production fields. Alleles of *L* gene have been broken by strains of *Pepper mild mottle virus* (PMMoV) but are believed to be durable against *Tobacco mosaic virus* (TMV). In contrast, *N'* is ineffective against TMV but exhibited resistance to all PMMoV strains we tested. To assess the potential risk of emergence of *N'*-breaking PMMoV, we established an Agroinfection system to screen the viruses with randomly mutated PMMoV CP for resistance breakage. PMMoV CP genes with random mutations (1.8 amino acid changes/clone in average; introduced using an error-prone PCR), were inserted into a PMMoV genome in a binary vector. Agroinfection of the PMMoV mutant library and immunodetection of CP identified 12 candidates for *N'*-breaking PMMoV CP out of 360 clones tested. In spite of the lack of resistance response and the clear detection of CP accumulation spread in the inoculated leaves, none of the candidate clones infected *N. sylvestris* systemically, suggesting that *N'*-mediated resistance is durable against PMMoV infection.

PS08-347

Increased expression of P3N-PIPO facilitate the cell-to-cell movement of *Clover yellow vein virus* in a *cyv1*-resistant pea

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There are two recessive resistance genes, *cyv1* or *cyv2*, controlling *Clover yellow vein virus* (ClYVV) in *Pisum sativum*. In pea lines carrying *cyv1*, an isolate of ClYVV, Cl-no30, was restricted in a single cell whereas another isolate, 90-1 Br2, overcame this resistance. We mapped the ClYVV element for the *cyv1*-resistance breaking by examining infection of the *cyv1* peas with chimeric viruses between Cl-no30 and 90-1 Br2, revealing that P3N-PIPO is involved in the resistance breaking. Then, how P3N-PIPO is involved in the resistance breaking was examined. P3N-PIPOs of other potyviruses were reported to be involved in cell-to-cell movement. We here confirmed that P3N-PIPO is also required for the cell-to-cell movement of ClYVV in infected plants, raising the possibility that Cl-no30 is defect in cell-to-cell movement in a *cyv1* pea and the 90-1 Br2 P3N-PIPO diminishes the defect of Cl-no30. To test this possibility, the GFP-tagged Cl-no30 infectious clone was biolistically inoculated into the *cyv1* pea with the plant expression vectors containing the *P3* or *P3N-PIPO* ORF from Cl-no30 and 90-1 Br2 under the 35S promoter. The virus movement was monitored with GFP fluorescence. As a result, additional expression of the P3N-PIPO derived from either Cl-no30 or 90-1 Br2 enabled Cl-no30 to move into adjacent cells but that of P3 rarely did. Susceptible peas infected with 90-1 Br2 accumulated more P3N-PIPO than did those infected with Cl-no30. These results suggested that the increased expression of P3N-PIPO facilitate the cell-to-cell movement of Cl-no30 in a *cyv1* pea, resulting in the resistance breaking.

PS08-348

A thioredoxin h protein from *Nicotiana benthamiana* is involved in the movement of *Bamboo mosaic virus*

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One of the downregulated genes in *Nicotiana benthamiana* after *Bamboo mosaic virus* <BaMV> infection identified by cDNA-amplified fragment length polymorphism <AFLP> technique was shown to participate in viral infection cycle. After retrieving the sequence by RACE technique, the protein product deduced from the full-length cDNA sequence has an ortholog to a thioredoxin h protein. Therefore, we designate this full-length clone *NbTRXh1*. To inspect how *NbTRXh1* is involved in the infection cycle of BaMV in *N. benthamiana* plant, we used the virus induced gene silencing <VIGS> technique to knock down the expression level of *NbTRXh1* in *N. benthamiana* plant and then inoculated BaMV. Results show that the accumulation of BaMV coat protein is increased in the knockdown plants at 5 dpi compared to that of control plants. However, we can not find any significant difference between the knockdown and control protoplasts at 24 hpi. Further, we also find out that BaMV is more efficient in infection and movement in *NbTRXh1*-knockdown plants than those in the control plants. In contrast, the accumulation of BaMV is reduced when this gene was transiently expressed in plants. Overall of these results suggest that the product of *NbTRXh1* gene may play a role in restricting BaMV movement rather in replication.

PS08-349

Determining the mechanism by which the p8 and p6.6 proteins from *Panicum mosaic virus* influence its intercellular movement in maize

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In the past three decades numerous studies were conducted to elucidate the mechanism of virus cell-to-cell movement in dicotyledonous species. However, similar research in monocotyledonous species has been more limited. *Panicum mosaic virus* (PMV) is an RNA virus and is the type member of the *Panicovirus* genus in the family *Tombusviridae*. PMV encodes two replication-associated proteins (e.g. p48 and p112) and four other proteins (e.g. p8, p6.6, p15, and the capsid protein). A previous study using mutants defective in expressing one of the four non-replication-associated proteins showed that the p8 and p6.6 proteins are likely involved in PMV movement between cells in its monocotyledonous host plant (Turina et al. 2000 Virology 266:120-128). We recently identified an Oklahoma strain of PMV (O-PMV) from a field *Panicum virgatum* (switchgrass) and produced a full length infectious clone of this virus. In addition, we cloned the p8 and p6.6 open reading frames (ORFs) and inserted them individually in front or behind a green fluorescent protein (GFP) ORF in a binary vector. We also developed a transient expression technique for these constructs in both dicotyledonous and monocotyledonous species using the Helios Gene Gun system and Tungsten M17 microcarrier. We will report on the intracellular location of these genes through their ectopic expression in cells of *Zea mays* cv. Oh28; in particular whether they localize to plasmodesmata. Initial findings indicate an unusual localization pattern dependent on the host species analyzed.

PS08-350

Evidence that SGT1 facilitates viral accumulation and induction of necrosis in *Tomato ringspot virus* infected plants

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Host genes play an important role in determining the outcome of plant-virus interactions. Plants inoculated with ToRSV-Rasp1 (a

severe *Tomato ringspot virus* isolate) display systemic necrosis at 21C. Symptoms are induced earlier at 27C but they are milder and plants eventually recover. Viral RNAs are detected earlier in infection at 27C than at 21C but accumulate to high levels later in infection at both temperatures. The role of host genes in this interaction is poorly understood. SGT1 (Suppressor of G2 allele of SKP1) is a multifunctional protein and is involved in R-gene mediated response, non-host resistance and programmed cell death. Prior to inoculation, SGT1 was found to be expressed at higher levels at 27C then at 21C. In addition, SGT1 was upregulated in response to virus infection at 21C. We investigated the effect of silencing SGT1 on viral RNA and protein accumulation and on the induction of necrosis at 21C. Following silencing of SGT1 using a virus-induced gene silencing (VIGS) approach, only 12% of ToRSV-Rasp1 inoculated plants displayed systemic necrosis at seven and fourteen days post-inoculation. In contrast 94% of control plants were necrotic at these time points. Viral RNA and coat protein accumulated at lower levels in SGT1-silenced plants in comparison to control plants, suggesting that SGT1 enhances virus accumulation. The reduced necrosis in SGT1-silenced plants may be a direct effect of the SGT1 silencing on the regulation of plant defense responses or it may be due to the lower levels of virus accumulation in these plants.

PS08-351

Cucumber mosaic virus (CMV) RNA3 transgenic *Nicotiana benthamiana* complement to express CMV -RNA1 and RNA2 systemically

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Cucumber mosaic virus (CMV) has a very broad host range and contains three single-stranded, genomic RNAs (RNAs 1-3). We have previously developed the CMV-based vector, which was an engineered RNA 2 to express a hetelorogous gene. We are now developing another system, where the CMV vector can be used together with transgenic plants expressing the CMV genes. In this study, we produced transgenic *Nicotiana benhtamiana* plants expressing CMV RNA3, which encodes the movement protein (3a) and the coat protein (CP). In these transgenic plants, the expression of CP was not detected as we expected, perhaps due to lack of RNA4 for CP. When we inoculated these transgenic plants with in vitro transcripts of CMV RNAs 1 and 2, we could of course observe CMV symptoms and detected a high level of accumulation of CMV-CP. To confirm whether we could produce a foreign protein in the transgenic plants using the CMV vector without CP-mediated resistance, the transgenic plants were inoculated with in vitro transcripts of CMV RNA 1 and RNA2:H1-GFP, whose 2b gene was replaced with the GFP gene. In the upper leaves of inoculated plants, GFP was clearly detected suggesting that the RNA3 transgenic plants can become a platform for foreign protein production using the CMV vector. In this system, we just used two viral RNAs instead of three, saving time and cost to produce virus-infected plants in a short period.This work was supported in part by grants from the Ministry of Economy Trade and Industry in Japan.

PS08-352

Identification of domains in p27 auxiliary replicase protein essential for its association with the endoplasmic reticulum membranes in *Red clover necrotic mosaic virus*

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Positive-strand RNA viruses require the host intracellular membranes for replicating their genomic RNAs. *Red clover necrotic mosaic virus* (RCNMV), a member of Dianthoviruses, has a bipartite genome consisting of RNA1 and RNA2. RNA1 encodes two N-terminally overlapping replication proteins p27 and p88. Our previous studies showed that p27 recruits RNA2 to the endoplasmic reticulum (ER) membranes via the interaction between the C-terminal region of p27 and a specific RNA element. In this study, we determined the domains and critical amino acids in p27 required for its association with, and targeting the ER membranes using C-terminally GFP-fused p27 (p27-GFP) that can support viral RNA replication in the presence of p88. Confocal microscopy and membrane flotation assays using an Agrobacterium-mediated expression system showed that a stretch of twenty amino acids in the N-terminal region of p27 is essential for the membrane association of p27, and that this domain alone is sufficient to target GFP to the ER membranes. We identified the amino acids in this domain required for the membrane association of p27 using alanine-scanning mutagenesis. We also found that this domain contains the amino acids not critical in the membrane association but are required for the formation of the RCNMV RNA replication complexes and negative-strand RNA synthesis. Our results extend our understanding of a multifunctional role of p27 in RCNMV replication.

PS08-353

The regulation mechanism of reactive oxygen species generation by calcium-dependent protein kinase

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The reactive oxygen species (ROS) generation, usually called the oxidative burst, was observed when the flagellin from Acidovorax avenae rice-avirulent strain treated to the cultured rice cells. ROS generation after recognition of the avirulent flagellin was strongly suppressed by Ca^{2+} chelating agents or kinase inhibitors. In order to investigate the Ca^{2+} dynamics during plant immune responses, yellow cameleon 3.6 was transiently expressed in cultured rice cells. When the avirulent flagellin was treated to the rice cells, Ca^{2+} concentration was rapidly increased, while a notable change was not observed by virulent flagellin treatment. These data indicated the possibility that ROS generation after recognition of the avirulent flagellin was regulated by calcium-dependent phosphorylation. Plants have calcium-dependent protein kinase (CPK) possessing protein kinase domain and EF hand motifs that may function in Ca^{2+}regulation. Among 29 rice *CPK* genes, 6 *CPK* genes were expressed after treatment of the avirulent flagellin. *OsCPK12* knock-down and knock-out mutants did not induce any ROS generation after treatment of the avirulent flagellin, suggesting that OsCPK12 regulated the ROS generation. Since it has known that Osrboh play a central role in ROS generation during biotic and abiotic stress, we examined interaction between OsCPK12 and Osrboh. Interaction experiment based on BiFC technology showed that OsCPK12 interacted with N-terminal domain of OsrbohA. These results proposed that the activity of OsrbohA might regulate via the N-terminal phosphorylation by OsCPK12.

PS08-354

Effect of rice RNA-dependent RNA Polymerase 1 (OsRDR1) on RNA silencing and small RNA regulation

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RNA silencing is a sequence specific gene regulation through RNA degradation, which is conserved across the fungal, animal

and plant kingdoms. The rice (*Oryza sativa*) mutant lines where *OsRDR1* was disrupted by the insertion of rice retrotransposon Tos17 was selected and further characterized for virus mediated RNA silencing and small RNA regulation. RNA silencing induction by particle bombardment was performed to investigate any effects of *OsRDR1* on RNA silencing with recombinant virus DNA/RNA in the mutant lines. The results showed that *OsRDR1* was required for RNA silencing mediated by *Brome mosaic virus* (BMV, ssRNA virus) but not for the silencing mediated by *Wheat dwarf virus* (WDV, ssDNA virus). Northern blot analysis of *Cucumber mosaic virus* (CMV) inoculated plants showed higher accumulation levels of CMV RNAs in the mutant lines than in the wild-type plants, indicating that *OsRDR1* plays an important role in antivirus defense. Furthermore, small RNA analysis showed that while the expression levels of some miRNAs were under the detection limit in the mutant lines, those of other miRNAs were increased depending on rice tissues, and that the mRNA level for targeted gene was inversely correlated with the expression level of each miRNA. Our observations suggest that *OsRDR1* is involved, either in a direct or indirect manner, in the biogenesis of these miRNAs.

PS08-355

Maize Ferredoxin-5 plays a negative role in *Sugarcane mosaic virus* infection

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Systemic virus infection in plants relies on complex molecular interactions between the invading virus and host proteins. Our previous works showed that *Sugarcane mosaic virus* (SCMV) HC-Pro could specifically interact with maize ferredoxin-5 (Fd V) in yeast and plant cells, then the role of Fd V in SCMV infection was further studied. The results showed that suppression of Fd V through virus-induced gene silencing (VIGS) in maize plants resulted in more severe mosaic symptom and enhanced the accumulation of SCMV viral coat proteins as well as viral genomic RNAs. However, transient over-expression of Fd V in maize protoplasts impaired SCMV multiplication. These data suggested that maize Fd V might play a negative role in SCMV infection.

PS08-356

Ultrastructural study of *Tomato yellow leaf curl virus* in the cells of host plants and the midgut epithelial cells of the insect vector, whitefly

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A geminivirus *Tomato yellow leaf curl virus* (TYLCV) is an important plant pathogen. Its infection to plants is strictly limited within the phloem. Various insects feed on the infected plants, but only a single species, a whitefly *Bemisia tabaci*, can transmit TYLCV from plant to plant. To obtain insight into the phloem tropism and the vector specificity, we analyzed ultrastructural localization of TYLCV in plant and insect cells by immunogold electron microscopy (IEM). When TYLCV was inoculated onto plants by the whitefly, the virus was accumulated only in phloem tissues, as reported. Nevertheless, when the virus was inoculated into *Nicotiana benthamiana* leaves by agrobacterium infiltration, the virus infected mesophyll cells and accumulated in an electron-dense matter within the cell nucleus. This indicates that the phloem tropism of TYLCV is not because of its inability to replicate in other cell types. TYLCV is transmitted by *B. tabaci* in a circulative, non-propagative manner. After *B. tabaci* was allowed to feed on infected plants, its digestive tract was submitted for IEM. The virus was localized only in electron-dense materials within vesicle-like structures found in the cytoplasm of midgut epithelial cells. These structures were observed at the descending and ascending midgut and the caecum. In contrast, no gold-labeling was detected in the midgut cells of a non-vector whitefly, *Trialeurodes vaporariorum*, after its feeding on infected plants. These results suggest that the vector specificity of TYLCV is due to its ability to enter *B. tabaci* midgut cells and inability to enter cells of other insects.

PS08-357

Evaluation of RNAi-mediated resistance offered to *Potato spindle tuber viroid* in transgenic *N. benthamiana* plants expressing different hairpin RNA constructs

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Viroids are small circular plant pathogenic RNAs. Unlike RNA viruses, viroids do not encode protein, and depend on host's transcriptional machinery for replication. As might be expected from their highly base-paired structure and RNA-RNA mode of replication, viroids have been shown to induce RNA silencing. Accumulation of viroid-specific small RNA (Vd-sRNA) has been reported upon infection in host plant. Previously, RNAi-mediated resistance against PSTVd infection has been observed in certain transgenic tomato lines expressing high levels of hairpin RNA-derived small viroid RNAs. Further, our deep sequencing data on small RNAs derived from PSTVd in tomato plants revealed certain hotspots on PSTVd molecule that tend to produce more small RNAs than other regions. With these backgrounds, we have produced transgenic *Nicotiana benthamiana* plants expressing hairpin constructs of near full length PSTVd and 21-nucleotide sequences derived from the Vd-sRNA hotspots of PSTVd. Resistance of the T2 plants expressing Vd-sRNA to PSTVd infection was analyzed by Northern hybridization. Some transgenic lines showed certain level of resistance. A good correlation has been observed between the level of resistance and the small RNA expression in transgenic lines. Higher levels of resistance were observed in transgenic plants expressing near full length PSTVd hairpin constructs rather than those expressing small RNA derived from hot spot.

PS08-358

A seed storage protein, PAP85, involved in early stage of replication of *Tobacco mosaic virus* and ER morphology change

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Plus-sense single-stranded RNA ((+)RNA) viruses induce the modification of the host intracellular membrane for assembly of membrane-bound virus replication complexes (VRCs). However, the host factors involved in this process remain largely unknown. We used microarray assay to screen the Arabidopsis gene(s) with response to infection of a (+)RNA virus, *Tobacco mosaic virus* (TMV), in the initial stage and identified an Arabidopsis gene, PAP85 (annotated as a seed storage protein), upregulated during initial TMV infection. Experiments with PAP85 knockdown and overexpression in pap85-RNAi plants suggested that PAP85 was involved in TMV accumulation. Co-expression of PAP85 and the TMV main replicase (P126) but not their expression alone in Arabidopsis protoplasts cells could modify the endoplasmic reticulum (ER) structure. PAP85 also induced ER modification in the presence of another 12S seed-storage protein (At1g03880, usually co-expressed with PAP85 during seed maturation). However, the induction of ER modification was protein specific, because co-expression of PAP85 and the ER marker could not modify the ER structure. Our data suggest PAP85 involved in TMV replication and TMV may hijack seed-storage proteins to modify the ER structure for replication. These also provide a starting point

for further investigation of the role of PAP85 in ER modification in plants.

PS08-359

Molecular characterization of *Potato spindle tuber viroid*-derived and non-related circular RNAs from dahlia

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Dahlia is a new natural host for viroid and supports the replication of *Potato spindle tuber viroid* (PSTVd) (Tsushima *et al* 2011). During the examinations on PSTVd infecting in dahlia, we have detected another single-stranded circular RNA molecules co-existing in the extracts. Some of them, by sequencing of RT-PCR products obtained by PCR primers derived from the upper central conserved region of PSTVd, were identified to be the deletion molecules of PSTVd, in which a stretches of sequence ranging 20-170 nucleotides were deleted. The other ones were also amplified coincidently by the same PCR primers but sequencing analysis revealed that they do not share any significant sequence similarity to known viroid species. They included several molecules with different sizes but shared partially identical units forming several chimeric structures.To investigate the biological importance of these viroid-like circular RNA molecules in plant, we have selected two PSTVd-derived deletion molecules of the size 188 and 304 nucleotides, and created the dimeric cDNA constructs. The analysis is now underway using tomato (cv. Rutgers) and dahlia to examine whether these deletion molecules have a potential to replicate by themselves. We are further conducting co-inoculation assay using *in vitro* transcribed deletion molecules and the intact PSTVd molecule to examine how these deletion molecules interact with the replication of intact PSTVd molecule.

PS08-360

Identification of the amino acids in Cap binding pocket of *Brassica rapa* eIF(iso)4E inducing the resistance against *Turnip mosaic virus*

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Turnip mosaic virus (TuMV) is one of the major viruses in Brassicaceae crops which belong to the genus *Potyvirus*. And eIF(iso)4E is well known for recessive resistance gene of potyvirus in many crops. To elucidate the key amino acids in the interaction between TuMV VPg and eIF(iso)4E, amino acids of eIF(iso)4E were mutated. Seven amino acids in cap binding pocket were chosen for the candidate amino acid that may play a role in the interaction of TuMV VPg. We demonstrated that a single amino acid mutation in cap binding pocket of Brassica eIF(iso)4E can abolish the interaction with TuMV VPg. eIF(iso)4E which has a mutation at each W49, W95 and K150 positions impaired in its interaction with VPg according to the yeast two hybrid analysis. BiFC assay result was also consistent with the yeast two hybrid data, as the signal was highly reduced in coexpression of eIF(iso)4E (W95L, K150L, W95L/K150E) and TuMV VPg. Complementation of an eIF4E knockout yeast strain by mutated eIF(iso)4E proteins showed that all eIF(iso)4E mutants were able to complement eIF4E of yeast. To find out if these mutations affect the susceptibility of Chinese cabbage, transformant analysis was performed. eIF(iso)4E W95L, W95L/K150E and susceptible wild type were over-expressed in susceptible Chinese cabbage. According to the TuMV screening result of T1 and T2 transformants, over-expression of the eIF(iso)4E mutants showed resistance to four TuMV strains (CHN2, 3, 4 and 5). Our results support that the mutations in eIF(iso)4E can engineer the broad spectrum TuMV resistance.

PS08-361

Seasonal dynamics and correlation studies of two viroids in two citrus cultivars

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Citrus exocortis viroid (CEVd) and *Hop stunt viroid* (HSVd or CVd-II) are two known citrus pathogens (documented in 1972 and 1995, respectively) that cause severe impacts on citrus industry in Taiwan. These two viroids usually co-infect citrus plants in Taiwan and their percentages of co-infection may reach up to fifty percent. The study of seasonal multiplicative dynamics and correlation between two viroids is necessary for understanding the ecology of CEVd and HSVd. For the quantitative investigation, the TaqMan® real-time RT-PCR assay was used to detect the presence and infection percentages of viroids in plant tissues, which were periodically sampled from seventeen natural hosts including eleven blood sweet oranges (*Citrus sinensis* var.) and six Murcott tangors (*Climentine* x *Citrus sinensis* Osbeck) in the middle Taiwan (Yunlin county). The results showed that both CEVd and HSVd unevenly distributed in their citrus hosts, and relatively higher concentration of viroids was found in twig barks. Correlation analysis between viroid titers and temperatures revealed that CEVd preferred warmer temperature whereas HSVd preferred cooler temperature. Interestingly, a positive correlation between two viroids is, however, only observed in Murcott tangors. The results presented in this study demonstrated that different viroids were likely adapted to different temperatures, and various citrus cultivars might show different interactive relationships between CEVd and HSVd. Further studies of long-term ecological survey will be conducted in the future.

PS08-362

Analysis of nucleo-cytoplasmic trafficking of the *Turnip crinkle virus* coat protein and its influence on plant defense responses

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Molecular research with *Arabidopsis* resistance to TCV demonstrated that TCV coat protein(CP) is an avr factor recognized by HRT(hypersensitive response to TCV) and also it plays as a viral suppressor of RNA silencing(VSR) on plant defense system. Here, we have investigated the interaction between HRT and CP or its natural mutants, D4N and P5S, which are escaped HRT recognition, and also their VSR activities using an *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana* plants, respectively. To assess the biological role of intracellular compartmentation of CP, we constructed fusion proteins between CP clones and fluorescent proteins, YFP:CP or CP:GFP, respectively. Both fusion CP proteins were detected in the cytoplasm, nucleus and probably nucleolus, but, interestingly, YFP:CP displayed an exclusive nucleolus distribution and accumulation in speckle-like structures. In addition, the recombinant CPs went on separate functions; CP:GFP only could but play a role of avr factor for HRT, while YFP:CP, as well as its distinct mutants(YFP:D4N, YFP:P5S), preserved its VSR ability albeit disappearing cell death response with HRT. In contrast to the low level of CP:GFP protein, immunoblot assay showed stable expression of variant N-terminal fused CP proteins, which maintained capacity of VSRs. Furthermore, changing the nucleocytoplasmic trafficking of the both CP fusion proteins using exogenous targeting signals revealed that recognition of CP by HRT is occurred in cytosol. Taken together, these results suggested that nucleo-cytoplasmic distribution of TCV CP is importance in initiating R-gene recognition, whereas its stability and localization of nucleolus are necessary to drive silencing suppression mechanism.

PS08-363

Identification of host proteins interacting with the capsid protein of *Odotoglossum ringspot virus*

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The capsid protein (CP) of an orchid infecting tobamovirus, *Odotoglossum ringspot virus* (ORSV), was previously shown to involve in virus long-distance movement. Substitution of Glu^{100} by Gly^{100} in the CP (CP^{E100G}) of ORSV abolished the systemic infection in *Nicotiana benthamiana* plant. According to the results of transmission electron microscopy, CP^{E100G} mutant could not produce uniform sized virions as wild type ORSV could. This data may partially explain the long-distance movement defect of ORSV CP^{E100G} mutant. However, the host proteins interacting with ORSV CP and involving in systemic infection are still unknown. To uncover the molecular interaction of host and ORSV CP, we created a cDNA library from the virus-infected *N. benthamiana* tissues and screened the CP-interacting host proteins through yeast two-hybrid system. Among 208 candidate clones from primary auxotroph selection, half of them gave positive results in β-galactosidase activity assay. So far, the cDNA inserts of 19 double positive clones were sequenced and analyzed using NCBI BLAST search within the database of GenBank. These clones could be classified into four groups including plant defense-related, metabolism-related, light-harvesting and energy synthesized-related genes. In addition, there are unknown-function *N. benthamiana* genes and a few transcription factors. In summary, several plant proteins interacting with ORSV CP were identified through yeast two hybrid screening. The further interaction and functional assays between ORSV CP and host factors will be investigated.

PS08-364

Induction of tobamovirus resistance in nontransgenic scions after grafting onto *NtTOM1* and *NtTOM3* silenced rootstocks

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TOM1 and *TOM3* are *Arabidopsis* genes which are required for tobamovirus multiplication. In the mutants of these genes tobamovirus cannot multiply. These genes are distributed in other plants including tomato, tobacco and melon. Silencing of both *NtTOM1* and *NtTOM3* in tobacco plants resulted in high resistance against several tobamoviruses (Asano et. al., 2005). RNA silencing is a novel mechanism of gene regulation by sequence specific RNA degradation and is involved in controlling endogenous gene expression and defense against invasive nucleic acids such as viruses. It is transmitted between scions and rootstocks through grafting in plants. In this study we examined the graft transmission of RNA silencing for conferring virus resistance to the non-transgenic scions grafted onto rootstocks in which both *NtTOM1* and *NtTOM3* were silenced (Sd1). Non-transgenic *Nicotiana tabacum* (cvs. Samsun and Xanthi nc) and *N. benthamiana* were used for grafting onto the Sd1 rootstocks. The leaves were detached from the scions 8 weeks after grafting and inoculated with several tobamoviruses including *Tobacco mosaic virus*, *Tomato mosaic virus* and *Wasabi mottle virus*. Then the virus accumulation was tested 16 days after inoculation by ELISA. As a result extremely low amount of virus was detected in grafted scions showing that the virus resistance was conferred. siRNA of *NtTOM1* and *NtTOM3* was detected in the scions as well as in the rootstocks. These results suggest that RNA silencing was induced in and virus resistance was conferred to the non-transgenic scions by grafting onto rootstocks in which two host genes were silenced.

PS08-365

***RCY1*-mediated resistance to *Cucumber mosaic virus* is regulated by LRR domain-mediated interaction with CMV(Y) following degradation of RCY1**

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RCY1, which encodes a CC-NB-LRR class R protein, confers the hypersensitive resistance (HR) response to a yellow strain of *cucumber mosaic virus* [CMV(Y)] in *Arabidopsis thaliana*. *Nicotiana benthamiana* (Nb) transformed with hemagglutinin (HA) epitope-tagged *RCY1* (*RCY1-HA*) also exhibited a defense response accompanied by HR cell death and induction of defense-related gene expression in response to CMV(Y). Following transient expression of *RCY1-HA* by agroinfiltration, the defense reaction was induced in *Nb* leaves infected with CMV(Y), but not in virulent CMV(B2)-infected *Nb* leaves transiently expressing *RCY1-HA* or CMV(Y)-infected *Nb* leaves transiently expressing HA-tagged *RPP8* (*RPP8-HA*), which is allelic to *RCY1*. This result suggests that *Arabidopsis RCY1*-conferred resistance to CMV(Y) could be reproduced in *Nb* leaves in a gene-for-gene manner. Expression of a series of chimeric constructs between *RCY1-HA* and *RPP8-HA* in CMV(Y)-infected *Nb* indicates that induction of defense responses to CMV(Y) was regulated by the LRR domain of RCY1. Interestingly, in CMV(Y)-infected *Nb* manifesting the defense response, the levels of both RCY1 and chimeric proteins harboring the RCY1 LRR domain were significantly reduced. Taken together, these data indicate that the *RCY1*-conferred resistance response to CMV(Y) is regulated by an LRR domain-mediated interaction with CMV(Y) and seems to be tightly associated with the degradation of RCY1 in response to CMV(Y).

PS08-366

Assessment of RNA exosome as a viral resistance factor

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As viral resistant mechanisms of plants, R gene-mediated resistance and RNA silencing are well known. By using RNA silencing, plants degrade viral RNAs and protect themselves. Plants have various RNA degradation machinery, besides RNA silencing, for instance, 5->3 or 3->5 exoribonucleases. Although RNA silencing was well studied as the defense machinery against viruses, whether or not the other RNA degradation mechanisms combat with viral RNAs or not is still uncertain. We focused our attention on RNA exosome, a widely conserved 3->5 exoribonuclease complex in eukaryote, and hypothesized that the RNA exosome degrades viral RNAs. To test this hypothesis, we tried to establish exosome knocked-down plant lines to infect plant viruses. We tried the new exosome knock-down method. It was impossible to infect viruses onto exosome knock-out mutants because almost all exosome null mutants showed lethal phenotypes. Then, we used artificial microRNAs [amiRNAs] strategy and expressed amiRNA only in mesophyll cells to avoid lethal phenotypes. As a result, the functional exosome knocked-down plants were successfully obtained. This method makes it possible to test whether the essential genes are related to virus virulence or not. Here, we report results of challenges of some viruses onto the exosome knock-down mutants.

PS08-367

The expression of miR398 and its target genes in BaMV transgenic *Nicotiana benthamiana* plants

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In *Arabidopsis*, miR398 and its target genes, Csd1, Csd2 and Ccs, are in response to some biotic and abiotic stresses. Both Csd1 and Csd2 are Cu-Zn superoxide dismutases (SODs) for catalyzing superoxide to hydrogen peroxide. Csd1 is cytosolic and Csd2 is in the chloroplasts. Ccs is a copper chaperone for all Cu-Zn SODs in *Arabidopsis*. However, little is known about their interaction with plant viruses. In this study, transgenic *Nicotiana benthamiana* plants expressing full-length cDNA of *Bamboo mosaic virus* (BaMV) were generated and two phenotypes were observed: asymptomatic (AS) and symptomatic (S) lines. Line S shows similar symptoms as those of BaMV-infected plants. To analyze the differential expressions of miRNAs in these two lines, miRNA array revealed that the expression of miR398 was highly induced in fully expanded symptomatic leaves of line S. The cloned full-length cDNA sequences of NbCsd1 shared 75% similarity with those of Csd1 in *Arabidopsis* and the predictive target site of miR398 was found in the 5' UTR of NbCsd1. However, microarray and real-time RT-PCR revealed the up-regulation of NbCsd1 in symptomatic leaves of line S. As expected, NbCsd2 expression was down-regulated in fully expanded leaves of both BaMV transgenic lines. Whether the NbCsd1 is the target of miR398 in *N. benthamiana* and the effect of miR398 on BaMV accumulation remain further investigation.

PS08-368

Host glycine rich protein 2 has a role in plant defense to virus infection

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Glycine-rich proteins (GRPs) are diversified in structure, expression pattern, modulation, subcellular localization and perform very distinct functions in planta. They involve in cell wall structure, cold and osmotic stresses, flowering time control and development. GRPs also confer defense against fungi and viruses as well as bacteria. In this study, using the 5' untranslated region (UTR) of *Bamboo mosaic virus* satellite RNA (satBaMV) as bait, we isolated the host GRP2 from evacuolated tobacco protoplast extract. In *Arabidopsis thaliana*, AtGRP2 is a cold-induced nucleo-cytoplasmic RNA-binding protein and requires for flower and seed development. The GRP2 of *Nicotiana benthamiana* shares approximately 60% identity with AtGRP2 in amino acid sequence. Electrophoretic mobility shift assay revealed that recombinant NbGRP2 binds to the 5' UTR of BaMV and satBaMV RNAs in vitro. Moreover, the accumulation level of BaMV RNA was higher in GRP2 silencing *N. benthamiana* than that of wild-type plant implying that GRP2 may play a role in plant defense mechanism to virus infection.

PS08-369

Transgenic expression of TMV capsid and movement proteins modulate plant basal defense and biotic stress responses in *Nicotiana tabacum*

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Plant viruses cause metabolic and physiological changes associated to symptomatic phenotypes of disease. Rapid viral replication in plant tissues involves the synthesis of large amounts of virus nucleic acids and proteins that in turn require re-direct host resources from normal cellular processes. Furthermore the effect called host gene shut-off compromises some aspects of plant physiology and broad-spectrum defense response. We used transgenic tobacco expressing a variant of *Tobacco mosaic virus* (TMV) coat protein (CPT42W) or movement protein (MP), and a hybrid transgenic line (MPxCPT42W) that co-expresses both proteins to study the plant response to individual viral proteins. Employing microarray analysis of MPxCPT42W plants and silenced mpxcpT42W* controls, we found that altered transcripts were mostly down regulated, suggesting a persistent shut-off due to MPxCPT42W expression. Next, we showed that MP was involved in ROS accumulation, reduction of total ascorbate and the expression of ROS scavenging genes. These effects were enhanced when both proteins were co-expressed. MP and MPxCPT42W plants showed increased levels of SA and SA-responsive genes expression. Furthermore, these effects were partially reproduced in *N. benthamiana* when GMP1 transcript was silenced. CPT42W seems to play a negative role in the defense response by reducing the expression of PR-1 and RDR-1. MP and MPxCPT42W transgenic expression promoted a recovery-like phenotype in TMV-RNA infections and enhanced susceptibility to *Pseudomonas syringae* and *Sclerotinia sclerotiorum*. It is evident that the mechanisms underlying disease susceptibility and tolerance or resistance depend on a complex regulatory network; and viruses are able to disrupt these fine tunings

PS08-370

Study of the involvment of the genes that encode the proteins SlGAL83 and TCTP in the infection of a susceptible host by *Pepper yellow mosaic virus*

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The genomes of most plant viruses code for only 4-10 proteins which are required to complete the infection cycle. For a successful infection, these viral proteins must interact with host factors, modulating metabolic pathways and coordinating a complex network pathogen favor. A subtractive library constructed from susceptible tomato plants infected by the potyvirus *Pepper yellow mosaic virus* (PepYMV) identified several genes which are putatively involved in the viral infection process, including those that code for the Translationally Controlled Tumor Protein (TCTP) and the tomato homologue of the *Saccharomyces cerevisae* Gal83 (SlGal83), a protein of the SNF1 complex. The objectives of this work were to study the roles of TCTP and SlGal83 during PepYMV infection in susceptible hosts. Transgenic tomatoes (cv. Moneymaker) silenced for these genes were generated and were inoculated with PepYMV. ELISA and qRT-PCR showed that non-transformed plants were infected, while silenced plants were ELISA negative and had reduced viral load. The subcellular localization of TCTP was analyzed. In healthy plants the subcellular localization of TCTP is cytoplasmatic and 48 hours after PepYMV infection, TCTP is relocated to the nucleus. To determine which PepYMV protein(s) promotes nuclear targeting of TCTP, each viral protein was coexpressed individually with pYFP-TCTP. Results showed that TCTP accumulates predominantly in the nucleus when co-infiltrated with CI and NIa.Together, the results of this work indicate that both TCTP and Sl-Gal83 play critical roles in the tomato-PepYMV interaction, being necessary for the establishment of a systemic infection

PS08-371

Viral infection dynamics and interference under the synergism between *Cucumber mosaic virus* and *Turnip mosaic virus*

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Co-infection of *Cucumber mosaic virus* (CMV) and *Turnip mosaic virus* (TuMV) exacerbate symptom severity on *Nicotiana benthamiana*. To examine spatial effects of the 2b protein (2b) of CMV in infection patterns, the CMV vectors expressing EGFP (EG) or DsRed2 (Ds) were used for inoculation onto *N. benthamiana*. CMV2-A1 vector (C2-A1 [A1]) has a functional 2b while CMV-H1 vector (C2-H1 [H1]) is 2b deficient. In a single infection, A1Ds highly accumulated in initial infection sites and showed extensive fluorescence in systemically infected leaves, whereas H1Ds disappeared rapidly from initial infection sites and could not spread efficiently in upper, non-inoculated leaf tissues. Furthermore, A1Ds could spread in the plants treated with salicylic acid (SA) after inoculation. The results suggested spatial effects of 2b against SA-mediated virus host resistance. In mixed infections with TuMV, we found new functions of 2b involved in unloading of CMV from vasculature into nonvascular tissues, and observed spatial interference (local interference) between CMV and TuMV at an early stage of mixed infection. The antagonistic interactions between CMV and TuMV were compromised by the synergy effects in subsequent infection dynamics. We believe that the phenomena observed in mixed infection of the two viruses provide novel insights into the relationships among RNA silencing suppressor, viral synergism, and interference.

PS08-372

Functions of the coat protein of *Potato virus A* are regulated by protein kinase CK2 phosphorylation and by a pathway involving cellular HSP70 and its co-chaperon CPIP

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We have identified an enzyme that phosphorylates the coat protein (CP) of *Potato virus A* (PVA, genus *Potyvirus*) as the protein kinase CK2 (Ivanov et al., 2003). Amino acid substitutions affecting the CK2 consensus sequence 242-TTSEED-247 in CP were introduced into a full-length infectious cDNA clone of PVA. Analysis of the viruses showed that e.g. ATAEED mutant could but AAAEED could not replicate. In another study we found that the heat shock protein 70 (HSP70) together with its co-chaperone CPIP (HSP40) regulates the functions of PVA CP (Hafren et al., 2010), which we believe is a novel mechanism to prevent premature particle assembly and to allow efficient viral RNA replication/translation to proceed. Our current aim is to study if a mechanistic link between CP phosphorylation and HSP70/CPIP-mediated regulation exists. Exogenic expression of wild type (wt) PVA CP inhibits viral gene expression but CK2 site mutants are less efficient in this function. Interestingly CPIP-mediated delivery of CP to HSP70 promotes degradation of PVA CP^{wt} when assayed in the absence of virus infection. Also, we show by silencing assays that the accumulation of PVA CP is affected by the available amount of CK2, HSP70 and CHIP.

PS08-373

Discovery and characterization of a novel calarvirus infecting potatoes in China

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A new carlavirus, tentatively named Potato virus H (PVH), was found on potato plants displaying mild symptoms in Hohhot, Inner Mongolia Autonomous Region. PVH was confirmed by genome sequencing, serological reactions, electron microscopy and host index assays. The PVH particles were filamentous and slightly curved, with a modal length of 570 nm. The complete RNA genomic sequences of two isolates of PVH were determined by Reverse transcription PCR (RT-PCR) and 5`Rapid amplification of cDNA ends (5`RACE) methods. Sequence analysis revealed that the PVH had a genomic organization typical of members of the genus *Carlavirus*, with a positive-sense single-stranded genome of 8410nt. It shared CP and replicase amino acid sequence identities of 38.2-57.7% with those of reported carlaviruses. Phylogenetic analyses based on the amino acid sequences of replicase and CP revealed that PVH formed a distinct branch, which is only distantly related to other carlaviruses. Western blot assays showed that PVH was not serologically related to other potato viruses (PVS, PVM and PoLV). Negative staining electron microscopic observation showedn that the PVH virion particles purified were filamentous and slightly curved, with modal length of 570 nm. Unlike other potato carlaviruses, PVH can systemically infect *Nicotiana glutinosa*, *Solanum tuberosum* and *Solanum lycopersicum*, but can not infect *Nicotiana tabacum* or *Nicotiana benthamiana*. All these results supported the classification of PVH as a novel species in the genus *Carlavirus*.

PS08-374

Two distinct sites are essential for virulent infection and support of variant satellite RNA replication in spontaneous *Beet black scorch virus* variants

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Spontaneous variants of *Beet black scorch virus* (BBSV) and its satellite RNA were generated from cDNA clones by serial propagation in *Chenopodium amaranticolor* and *Nicotiana benthamiana*. Inoculation with recombinant RNAs synthesized in vitro revealed BBSV variants with divergent infectious phenotypes that affected either symptom expression or replication of satellite RNA variants. Sequence alignments showed a correlation between the phenotypes and distinct BBSV genomic loci in the 3' UTR or in the domain encoding the viral replicase. Comparative analysis between a virulent variant BBSV-m294 and the wild type (wt) BBSV by site-directed mutagenesis revealed that a single nucleotide (nt) substitution of a uridine to a guanine at 3477 nt in the 3' UTR was responsible for significant increases in viral pathogenicity. Gain-of-function analyses demonstrated that the ability of the BBSV variants to support replication of variant satRNAs was mainly determined by amino acid 516 in the P82 replicase. In this case, an arginine substitution for a glutamine residue was essential for high levels of replication, and the alterations of other residues surrounding position 516 in the wt BBSV isolate led to only minor phenotypic effects. These results provide evidence that divergence of virus functions on pathogenicity and supporting parasitic replication can be determined by a single genetic site, either a nucleotide or an amino acid.

PS09-375

Extracellular apyrase (ecto-ATPase) regulates the peroxidase-catalyzed apoplastic oxidative burst in cowpea (*Vigna sinensis* Endl.): implication in nonhost resistance

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Supprescins A and B from *Mycosphaerella pinodes* are glycopeptide suppressors for defenses, but they act as common elicitors on non-host plants. Recently, one target for the supprescins is proposed to be cell wall-associated apyrases (ecto-ATPases). Indeed, they can inhibit the ATP-hydrolyzing activity in cell walls of pea, but stimulates the activity of non-host plants such as cowpea. In this study, cowpea was used to analyze the role of ATP hydrolysis in non-host responses. Purified supprescins induced biphasic generation of SOD-sensitive superoxides ($O_2^{\cdot-}$). Pharmacological studies with inhibitors and antioxidant enzymes showed that the $O_2^{\cdot-}$ generation largely depends on an extracellular peroxidase(s) rather than a membrane-bound NADPH oxidase, because it was sensitive to salicylhydroxamic acid (SHAM). Since NADH inhibitor I-1 completely reduced the $O_2^{\cdot-}$ generation, the oxidation of apoplast NADH (as an electron donor) is likely involved in the peroxidase-catalyzed $O_2^{\cdot-}$ generation. Interestingly, the $O_2^{\cdot-}$ generation was accompanied by a production of a low molecular weight anti-fungal (yet-unidentified) compound(s), which suppresses fungal penetration from appressoria. Silencing of *VsNTPase1* encoding the cowpea cell wall-associated apyrase (ecto-ATPase) attenuated the $O_2^{\cdot-}$ generation, allowing to be susceptible to infection by a non-pathogenic fungus. Experiments with adenine nucleotide analogues revealed that ADP enhanced $O_2^{\cdot-}$ generation induced by the suppressins. Moreover, a non-hydrolysable ADP[β]S alone evoked SHAM-sensitive $O_2^{\cdot-}$ generation. Taken together, these results indicate that cell wall-associated apyrase/ecto-ATPase spatially regulates the peroxidase-catalyzed apoplastic oxidative burst through the hydrolysis of adenine nucleotides, substantially sustaining non-host resistance of cowpea.

PS09-376

Defense-related LsGRP1 protein may link to cell wall pectin and involve in disease resistance regulation *via* protein-protein interaction

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Induced resistance is an enhanced defensive state of plants against different kinds of stresses from the environment. *LsGRP1* (named for *Lilium* Stargazer Glycine-Rich Protein 1) is a defense-related gene differentially expressed in salicylic acid-treated lily plants with increased resistance against *Botrytis elliptica*. Transient expression of *LsGRP1* in *Nicotiana benthemiana* could reduce symptoms caused by *Botrytis cinerea*. LsGRP1 expression specifically in leaf tissues and accumulation mainly in epidermal and vasculature cells were confirmed by western blot analysis and immunolocalization assay, respectively. LsGRP1 could be extracted with sodium dodecyl sulfate solution, but not with phosphate buffer saline. Addition of pectinase in the extraction buffer significantly increased the recovery of LsGRP1 from lily leaves, suggesting that LsGRP1 is localized in the cell wall *via* covalently binding to the pectin. Many cell wall-localized defense-related proteins were reported to regulate disease resistance *via* interaction with certain cell wall proteins. Thus, the putative interacting protein of LsGRP1 is investigated by co-immunoprecipitation to explore the action of LsGRP1 in the induced resistance of lily.

PS09-377

SignWALLing: Signals derived from Arabidopsis cell wall activate specific resistance to pathogens

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The traditional view of the cell wall as a passive barrier has evolved to a new concept that considers the wall as a dynamic structure that regulates both constitutive and inducible plant defence responses. The activation of plant innate immune system can be triggered by microbe-associated molecular patterns (MAMPs) from the pathogens, but also by damaged-associated molecular patterns (DAMPs), that are molecules released from plant cell walls upon pathogen infection or wounding. In line with this putative function, we have identified novel regulators of *Arabidopsis* resistance to necrotrophic fungi that may also be involved in the control of cell wall structure. To further characterize the function of cell wall on the regulation of immune responses, we have performed a biased resistance screening of putative/characterized primary/secondary *Arabidopsis thaliana* cell wall mutants. In this screening we have identified 20 mutants with altered susceptibility/resistance to at least one of the following pathogens: *Plectosphaerella cucumerina*, *Ralstonia solanacearum*, *Hyaloperonospora arabidopsidis* and a powdery mildew fungus. Expression analyses of the immune response genes in the selected mutants revealed a complex regulation of the defensive responses in these mutants. We found that cell wall extracts from some of the selected mutants conferred resistance to particular pathogens when applied to wild-type plants, further suggesting the presence of DAMPs in the wall extracts of these mutants. These data together with those obtained in the characterization of the cell wall from the selected mutants suggest a putative interconnection between cell wall structure/composition and resistance/susceptibility to pathogens.

PS09-378

Plant response to danger signals

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Plants are continuously tormented by various stresses from the environment. These stresses, for example diseases caused by pathogens, greatly reduce plant growth, distribution and productivity. However, plants are not defenseless: immobility has forced the development of various sophisticated defense mechanisms triggered as a response to environmental signals. These measures alter plant metabolism aiming to ensure the survival of the plant. In the case of pathogens, rapid detection and following defense activation is essential. Pivotal element in the innate immunity system of plants is the recognition of conserved, pathogen-derived molecules, called PAMPs (pathogen associated molecular patterns) that can be present in both pathogenic and non-pathogenic micro-organisms. Importantly, plants can also recognize damage to self: endogenous molecules such as fragments of plant cell wall. These damage associated molecular patterns, DAMPs, can be released as a result of microbial enzyme action or herbivore attack (wounding) and constitute danger signals that trigger plant defense responses similarly to PAMPs. DAMP-triggered defense signaling has an important role for example in defense activation against many necrotrophic/hemibiotrophic pathogens such as *Pectobacterium carotovorum* that breaks down plant tissue by secreting cocktail of extracellular enzymes as their pathogenicity strategy. We aim at finding new molecular components of DAMP-triggered defense signaling by using oligogalacturonide (OG) elicitors as DAMP model to screen T-DNA mutagenized Arabidopsis seed pool for insensitive mutants. Our preliminary results indicate that several of the OG-insensitive mutant lines have altered pathogen tolerance phenotype. Increased or decreased tolerance has been observed against *Pectobacterium* sp., *Pseudomonas syringae* and *Botrytis*

cinerea.

PS09-379

Infection inhibitor(s) generated in the cell wall preparation from *Pisum sativum* L.

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A glycoprotein elicitor from *Mycosphaerella pinodes* was found to induce rejection reaction to the pathogen and generation of infection-inhibiting activity on uninjured pea tissues (*Pisum sativum* L. cv. Midoriusui) within 1 h after treatment (Yamamoto et al. 1986). Moreover the infection-inhibiting activity was also found in pea cell-wall preparation treated with the elicitor (our unpublished data). Thus the generation of infection-inhibitor is one of rapid defense responses against invading pathogens. By purification with TLC and HPLC, at least, two active compounds were detectable in the fraction from the elicitor solution, and one of them was identified as dihydromaleimide (DHM). HPLC analysis indicated that 2.7 nmol/gFW (pea tissues) DHM accumulated within 1 h after treatment with *M. pinodes*-elicitor or 1 mM CuSO4. Dihydromaleimide (above 50 μM) inhibited penetration from appressoria of *M. pinodes*, although it scarcely affects germination and appressorial formation. Penetration by several pathogenic fungi such as *Colletotrichum orbiculare*, *C. destructivum* and *Alternaria alternata* were also inhibited by treatment with above 5 μM DHM. Interestingly, 0.5~5 μM DHM alone, which could not block the penetration, induced rejection reaction at 24 h after treatment and stimulated the transcriptional activation of several defense-related genes at 3 h after treatment in *Medicago truncatula* and *Arabidopsis thaliana* Col-0, suggesting that DHM was also able to induce resistance. Based on these findings, we discuss the role of DHM in defense response and the application to cultivation.

PS09-380

The apoplastic oxidative burst and induced extracellular defense: production of an anti-fungal compound(s) in the extracellular space of cowpea leaves challenged with the fungal elicitor

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In cowpea leaves challenged with the fungal elicitor from a pea pathogen *Mycosphaerella pinodes*, the apoplastic oxidative burst; i.e. superoxide production is induced through the oxidation of NADH (as an electron donor) by an extracellular peroxidase(s), substantially contributing to non-host-resistance of cowpea (see a poster by Tanaka, K. *et al.*). In this study, to clarify the role of inducible defense(s) in the extracellular space of cowpea, an *in vitro* assay with ethanol-killed onion epidermis and phytopathogenic fungus including *M. pinodes* and *Colletotrichum orbiculare* was carried out to examine whether an anti-fungal compound(s) is newly generated in cowpea leaves. When leaf discs from epidermis-peel-off cowpea leaves were floated on the elicitor solution, O_2^- was abundantly released into the test solution within 15 min, accompanied by a production of anti-fungal (yet-unidentified) compound(s) suppressing the penetration from appressoria but scarcely affects spore germination. Dilution-end-point analysis for the extracellular solution showed that the putative compound(s) was effective even at one hundred-fold dilution. The compound(s) was a hydrophilic and heat-stable (95°C for 10 min). Separation with ultrafiltration and subsequent HPLC analysis revealed that compounds less than 500 Da were responsible for the penetration inhibition. Taken together, it is likely that cowpea leaves respond to produce an infection-inhibitor(s) extracellularly upon the fungal elicitor-treatment. Further purification and characterization of the compound(s) are now underway to understand the role of the extracellular defense in non-host resistance of cowpea.

PS09-381

Sub-cellular dynamics of beta-1,3-glucanases during stress response

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Degradation of callose at plasmodesmata (Pd) is mediated by beta-1,3-endoglucanases (BGs). We showed previously that Arabidopsis Pd-associated BG (AtBG_pap) is a constitutively expressed GPI-anchored extracellular protein, which increases Pd size exclusion limit (SEL) by degradation of callose at Pd. Unlike AtBG_pap, two Arabidopsis pathogenesis related BGs (PR-BGs), AtBG2 and AtBG3, that are highly induced by biotic stresses, are predicted to be free extracellular proteins. In tobacco, PR-BGs were shown to increase Pd SEL and enhance virus spread. In order to determine the cellular mechanism of PR-BGs functioning in stress conditions, we monitored their targeting during various stresses. We show that over-expression of AtBG2-GFP results in retention of the protein in ER, and that its secretion to cell wall is induced only in stress conditions involving cell death. Secreted AtBG2 is not specifically enriched at Pd. The secretion of AtBG2 is highest in living cells that surround the necrotic lesion induced by high concentrations of salicylic acid (SA) or *Botrytis cinerea* infection, and decreases in cells farther from the lesion. We conclude that induction of PR-BGs during stress is a two-component process involving accumulation in the ER and subsequent localized secretion. We also show that it is the catalytic domain of AtBG2 that controls its conditional secretion. Currently, other candidate stress responsive BGs from Arabidopsis are being characterized.

PS09-382

Activities of 9-lipoxygenase in controlling plant defence and cell wall integrity

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Plant oxylipins are a class of lipid signalling molecules with a critical role in protecting plants against pathogen attack. Recent data demonstrated the participation of the 9-LOX and alpha-DOX oxylipin pathways in plant defence. Studies with mutants deficient in oxylipin production indicated that the 9-LOX and alpha-DOX oxylipins participate in the three layers of defence -pre-invasion, apoplastic, systemic defence- triggered by Arabidopsis to prevent Pseudomonas syringae pv tomato DC3000 infection. In these responses, oxylipins were found to act as regulators of oxidative stress and hormone homeostasis. Our studies also showed high 9-LOX and alpha-DOX activity in roots of untreated plants, where these pathways participate in plant defence mechanisms against root pathogens, a process that remains poorly understood. Further knowledge on the mode of action of oxylipins was obtained by characterization of noxy mutants (non-responding to oxylipins), which are deficient in signalling the response to the 9-LOX-derivative, 9-hydroxyoctadecatrienoic acid (9-HOT). In accordance with the role of 9-LOX in plant defence, we found that a high percentage of the noxy mutations showed enhanced susceptibility to virulent Pseudomonas. Moreover, in these studies we found that noxy mutants were altered in the signalling pathway that is activated after cellular damage to maintain cell wall integrity. These results support the participation of 9-LOX, and of the derivatives produced trough this oxylipin pathway, in inducing a sustained

defence response. The location of noxy mutations at loci encoding mitochondrial proteins indicated the participation of this organelle in establishment of robust immunity.

PS10-383

The vascular pathogen *Verticillium longisporum* exploits a jasmonic acid-independent COI1 function in roots to enhance disease symptoms in *Arabidopsis thaliana* shoots

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The soil-borne vascular pathogen *Verticillium longisporum* causes reduced shoot growth and early senescence in *Arabidopsis thaliana*. Analyses of plant mutants in the jasmonic acid (JA)-dependent signaling pathway revealed that disease symptoms are less pronounced in plants lacking the receptor of JA, CORONATINE INSENSITIVE 1 (COI1). Initial colonization of the roots was comparable in wild-type and *coi1* plants and fungal DNA accumulated to almost similar levels in petioles of wild-type and *coi1* plants at 10 days post infection. At late disease stages the number of plants with microsclerotia was reduced in *coi1*, indicating that completion of the fungal life cycle is impaired. Contrary to the expectation that the hormone receptor mutant *coi1* should display the same phenotype as the corresponding hormone biosynthesis mutant *dde2*, *dde2* plants developed wild-type-like disease symptoms. Induction of marker genes of the JA and the JA/ethylene defense pathway in wild-type petioles but not in *dde2* petioles indicated absence of fungal compounds that would activate the known COI1-dependent signal transduction chain. Grafting experiments revealed that the susceptibility-enhancing COI1 function acts in the roots. Moreover, we showed that the *coi1*-mediated tolerance is not due to the hyper-activation of the salicylic acid pathway. In combination with previously reported results on the *Fusarium oxysporum*/*Arabidopsis* interaction, this study points at a conserved strategy of two vascular pathogens to weaken the host tissue through an unknown JA-Ile-independent but COI1-dependent mechanism in the roots which influences disease-promoting processes in the shoot.

PS10-384

Compensatory functions of salicylic acid and MAPK signaling in effector-triggered immunity

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Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are triggered by recognition of conserved microbial features called microbe-associated molecular patterns and specific pathogen effectors, respectively. We reported that some cases of PTI and ETI extensively share signaling machinery but the common network is used differently: synergistic relationships among signaling sectors are evident in PTI, which may represent signal amplification; compensatory relationships dominate in ETI, explaining the robustness of ETI. To further investigate network properties of plant immunity, we analyzed expression profiles in WT and the salicylic acid (SA) biosynthesis mutant *sid2* during PTI and ETI. We found that regulation of a large number of genes were strongly SA-dependent in PTI; however, some of the genes including a canonical SA marker gene, *PR1*, are regulated in an SA-independent manner in ETI. A MAP kinase, MPK3, is activated during both PTI and ETI but the timing of activation is different: shorter than 1 hour in PTI; up to 10 hours in ETI. We found that prolonged MPK3 activation by inducible expression of a constitutively active form of an MPK3-activating MAPKK, $MKK4^{DD}$, led to induction of *PR1* in an SA-independent manner. These results suggest that the prolonged MPK3 activation during ETI can transcriptionally regulate the genes typically regulated by SA independently of SA and that this compensation could contribute to a robustness property of the ETI network. Thus, PTI and ETI use MPK3, but with different timing. This is a specific example of the two responses using common machinery in different ways.

PS10-385

The role of plant hormones in the interaction between rice roots and nematodes

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Rice is one of the most important crop plants worldwide and an excellent model system for monocotyledonous plants. Estimates of annual yield losses due to plant-parasitic nematodes on this crop range from 10 to 25% worldwide. The two agronomically most important nematodes attacking rice are the rice root knot nematode *Meloidogyne graminicola* and the migratory root rot nematode *Hirschmanniella oryzae*. These two nematodes have very different lifestyles, and comparing the rice defence system upon infection with these pathogens can provide important insights into general and specific defence strategies of the rice plant. Recently, we have analyzed the local response of roots upon infection with these nematodes using high throughput RNA sequencing (mRNA-Seq). Results showed that several major hormone pathways are influenced by nematode infection. While migratory nematode infection causes an induction of biotic stress-related genes early in the infection, the root knot nematodes appear to strongly suppress defense-related hormone pathways, like the salicylic acid and ethylene pathways. Next to this local defence suppression, a systemic down regulated of plant defence-related genes was also demonstrated by qRT-PCR on shoots of root knot nematode infected plants. Experiments with pharmacological treatments and rice mutants revealed that the jasmonate pathway is a key player in systemically induced defence against root knot nematodes, and that this pathway is antagonized by the brassinosteroid pathway to promote susceptibility of the rice root. On the other hand, salicylic acid seems to be a potent inducer of defence against root rot nematodes.

PS10-386

***Moniliophtora perniciosa-Solanum lycopersicum* interaction in tomato hormonal mutants**

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The basidiomycete *Moniliophthora perniciosa* (C-biotype) causes witches' broom disease in cacao (*Theobroma cacao*), but isolates of the S-biotype can infect Solanaceae species, including tomato (*S. lycopersicum*). The miniature tomato Micro-Tom was used as a model to investigate the role of hormones in pathogenesis. The mutants *dgt* (reduced auxin sensitivity); *not* (ABA deficient); *epi* (ethylene overproducer); *Nr* (ethylene insensitive); *pro* (gibberellin constitutive signaling); *cu3* (reduced brassinosteroid sensitivity); *jai1* (reduced jasmonate sensitivity); and the transgenic line *35::AtCKX2* (reduced endogenous cytokinin levels), all in the Micro-Tom background, were inoculated with basidiospores from the S- or C-biotype. All mutants inoculated with the S-biotype developed typical symptoms in tomato (stem thickening and lateral overgrowth), but *not* and *pro* presented a higher number of symptomatic plants, indicating that low levels of ABA and constitutive response to gibberellins increased susceptibility. Ethylene appeared to affect pathogenesis, as inoculated *Nr* showed

significantly thicker stems, whereas *epi* displayed a subtle decrease in hyperplasia. All mutants inoculated with the C-biotype did not exhibit symptoms, but all plants presented reduced height and growth. Expression of 14 genes associated with defense response was evaluated by RT-qPCR in Micro-Tom inoculated with S- or C-biotype up to 720h. In plants inoculated with the S-biotype, 11 genes significantly accumulated more transcripts, especially 48h after inoculation. Similarly, inoculation with the C-biotype significantly increased transcripts for 13 genes, peaking at 72h after inoculation, especially *PR1a* and *PR1b*. The contrasting compatibility presented by C- and S-biotypes reflected quantitative and kinetic differences in gene activation of the same response repertoire.

PS10-387

Profiling of specific proteins induced in Japanese birch plantlet treated with salicylic acid or azelaic acid

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Salicylic acid (SA) is known to induce systemic acquired resistance (SAR) in plants. In addition, a recent study demonstrated that azelaic acid (AZA) is a possible translocated signal molecule to induce SAR in *Arabidopsis thaliana*. In the present study, therefore, profile changes in specifically expressed and increased/decreased proteins in the Japanese birch plantlets treated with SA or AZA. The plantlets were treated with SA aqueous solution or AZA in MES buffer. Intact (C1), wounded and ultra-pure-water-infiltrated ($C2_{SA}$) or wounded and MES-buffer-infiltrated ($C2_{AZA}$) plantlets were prepared, respectively. Two days after the treatments, each plantlet was collected, and protein samples were prepared from them. The samples were subjected to 2D electrophoresis, in-gel digestion, and LC/MS/MS. The numbers of total protein spots in C1, $C2_{SA}$, and T_{SA} were 718, 739, and 763, respectively. The numbers of the protein spots specifically expressed in C1, $C2_{SA}$, and T_{SA} were 47, 34, and 23, respectively. Five specifically expressed, 3 significantly increased, and 3 significantly decreased proteins in T_{SA} were identified by sequence tag method as follows: malate dehydrogenase, SDH1-1;ATP binding / succinate dehydrogenase, phosphoglycerate kinase, diaminopimelate decarboxylase, arginase, chorismate mutase, peptidylprolyl isomerase (cyclophilin), aminopeptidase, and two hypothetical proteins. These proteins are considered to be involved in energy production, metabolism, and protein synthesis for SAR induction in birch plantlets. The numbers of total protein spots in C1, $C2_{AZA}$, and T_{AZA} were 788, 809, and 861, respectively. The numbers of the protein spots specifically expressed in C1, $C2_{AZA}$, and T_{AZA} were 13, 18, and 26, respectively.

PS10-388

Rice WRKY62 is a positive regulator of SA-pathway-mediated regulation of diterpenoid phytoalexin synthesis genes in rice

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Rice WRKY62 is a transcription factor in the salicylic acid (SA) signaling pathway in rice and its gene is transcriptionally regulated by WRKY45, one of key transcription factors in the rice SA pathway. Previously, it was reported that WRKY62 interacts with Xa21, a receptor-like kinase involved in *Xanthomonas oryzae pv. oryzae (Xoo)* recognition, and *WRKY62* overexpression enhanced *Xoo* susceptibility, suggesting that WRKY62 is a negative regulator of defense. Here, we functionally characterized WRKY62 focusing on its role in the SA-pathway and showed that it plays a positive role in the regulation of defense genes and disease resistance. *WRKY62* was induced in temporal patterns similar to those of *WRKY45* after *M. oryzae* infection or BTH treatment. WRKY62 showed active transcriptional repression activity in a transient reporter gene assay in rice protoplasts, whereas WRKY45 is a transcriptional activator. Microarray analyses in *WRKY62*-knockdown rice (*WRKY62*-kd) revealed that BTH-induced expression of the genes for biosynthesis of antimicrobial diterpenoid phytoalexins; momilactones, phytocassanes, and oryzalexins, and several *PR* genes were dependent on WRKY62. In addition, *WRKY62*-kd rice plants were more susceptible to *M. oryzae*. These results indicate that WRKY62 is a positive regulator of SA-pathway-mediated defense program in rice. Overexpression of *WRKY45* induces a strong pre-invasive defense to *M. oryzae*, to which the diterpenoid phytoalexins and PR proteins presumably contribute. Investigation of molecular mechanisms underlying transcriptional regulation of the defense genes by WRKY45 and WRKY62 is under way.

PS10-389

Genetic dissection of jasmonate-flagellin antagonism

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Accumulating evidence suggests that plant hormones play an integral role in plant innate immune responses and are capable of suppressing pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) in plants. Clear examples are jasmonate (JA)-flg22 and brassinolide-flg22 antagonisms. However, the molecular mechanisms underlying the hormone-PTI interactions remain elusive. To identify regulatory factors in hormoe-PAMP antagonism, we sought to identify key components in JA-flg22 antagonism using a genetic approach. We have previously reported that coronatine (COR), a phytotoxin secreted by pathovars of *Pseudomonas syringae* that mimics JA-Ile in activating JA signaling, suppressed expression of flg22-induced genes, including CYP71A12. We performed a genetic screen to look for mutants defective in COR-mediated suppression of flg22-triggered signaling, by monitoring GUS expression in a CYP71A12p:GUS reporter line. Approximately 30 mutants with strong suppression of CYP71A12p:GUS expression were isolated, including 10 coi1 alleles and one myc2 allele, two key genes in JA signaling. Molecular cloning and detailed characterization of the remaining mutants are currently in progress.

PS10-390

Translocation of phospholipase A2α to apoplasts is modulated by developmental stages and bacterial infection in Arabidopsis

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Phospholipase A2 (PLA2) hydrolyzes phospholipids at the sn-2 position to yield lysophospholipids and free fatty acids. Of the four paralogs expressed in Arabidopsis, the cellular functions of PLA2α in planta are poorly understood. The present study shows that PLA2α possesses unique characteristics in terms of spatiotemporal subcellular localization, as compared with the other paralogs that remain in the ER and/or Golgi apparatus during secretory processes. Only PLA2α is secreted out to extracellular spaces, and its secretion to apoplasts is modulated according to the developmental stages of plant tissues. Observation of PLA2α-RFP transgenic plants suggests that PLA2α localizes mostly at the Golgi apparatus in actively growing leaf tissues, but is gradually trans-located to apoplasts as the leaves become mature. PLA2α promoter::GUS assays show that PLA2α gene expression is controlled in a developmental stage- and tissue-specific manner. PLA2α gene expression is also regulated by photoperiod: in contrast to long-day conditions, in short-day conditions the levels of PLA2α expression significantly decrease and its secretion to apoplasts in mature leaves is not evident. When *Pseudomonas syringae* pv. *tomato* DC3000 carrying the avirulent factor avrRpm1 infects the apoplasts of host plants, PLA2α rapidly trans-locates to the apoplasts where bacteria attempt to become established. These results suggest that PLA2α may function in plant

defense responses at apoplasts where host confronts with invading pathogens.

PS10-391

Proteome and transcriptome analysis of wound-induced accumulation of salicylic acid in WIPK/SIPK-suppressed plants

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Salicylic acid (SA) is a signaling molecule which plays a key role in plant resistance to pathogens. Accumulation of SA is abnormally induced by wounding in the tobacco plants with suppressed expression of WIPK and SIPK, two pathogen- and wound-induced mitogen-activated protein kinases. SA accumulation started between 12 and 15 hr, and peaked at 15 hr after excision of leaf discs (wounding). Wound-induced accumulation of SA was inhibited by cycloheximide (CHX), a protein synthesis inhibitor, in a dose-dependent manner. To clarify the periods required for SA accumulation, leaf discs were first floated on water for the specific periods and then transferred to CHX solution. As a result, four-hr incubation on water was sufficient for SA accumulation. Unexpectedly, when leaf discs were transferred to CHX solution after incubation on water for 6-hr or more, SA accumulation was enhanced several fold. Analyses on spatial pattern of SA accumulation revealed that SA is mainly accumulated in the wounded region when leaf discs were floated on water throughout, but in the unwounded region when leaf discs were transferred to CHX from water, suggesting that, when floated on CHX, protein synthesis is inhibited only in the wounded region, but not in unwounded region. In fact, infiltration of CHX into unwounded region completely abolished SA accumulation. To clarify mechanisms underlying wound-induced and CHX-enhanced SA accumulation, proteins and transcripts which are differentially accumulated in WIPK/SIPK-suppressed plants were identified by proteome and transcriptome analyses. The mechanisms of SA induction by wounding in WIPK/SIPK-suppressed plants will be discussed.

PS10-392

VOZ governs abiotic and biotic stress responses in *Arabidopsis*

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VOZs(Vascular plant One-Zinc-finger proteins), the plant-specific one-zinc-finger type DNA binding proteins, are highly conserved in land plant evolution. Although VOZ2 protein has been demonstrated to bind in vitro to GCGTNx7ACGC in the V-PPase promoter, their physiological functions remain to be elucidated. Here, we provide insight into the regulatory mechanism by which VOZs modulate stress responses in Arabidopsis. Considerable stress-responsive genes were expressed in the *voz1voz2* double mutant, even under normal growth conditions. In addition, the *voz1voz2* double mutant increased cold tolerance with or without cold acclimation. Furthermore, tolerance to drought stress was significantly greater in *voz1voz2*, although resistance to a fungal pathogen, *Colletotrichum higginsianum*, and a bacterial pathogen, *Pseudomonas syringae*, were severely impaired. Thus, loss-of-function of VOZs conferred increased abiotic tolerance and biotic stress susceptibility simultaneously. During cold-exposure, both the mRNA expression levels of *VOZ1* and *VOZ2* and *VOZ2* amount gradually decreased. In *voz1voz2*, expression of the ABA-inducible transcription factor CBF4 was significantly upregulated even under normal growth conditions, despite the endogenous content of ABA being significantly unaltered, suggesting that VOZs negatively affect CBF4 upregulation in an ABA-independent manner. These results suggest that VOZs function as a negative regulator of the abiotic stress responsive pathway and positive regulator of the biotic stress responsive pathway, controlling the adaptation of plants to various stress conditions in Arabidopsis.

PS10-393

The nuclear ubiquitin proteasome regulates WRKY45 function in a dual mode in the rice defense program

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WRKY45 is one of key transcription factors in the salicylic acid (SA) signaling pathway in rice and plays an essential role in BTH-induced resistance to rice blast (*M. oryzae*) and bacterial leaf-blight (*Xanthomonas oryzae pv. Oryzae, Xoo*) diseases. Here, we show that WRKY45 is regulated by ubiquitin-proteasome system (UPS) in a dual mode. A treatment of myc:WRKY45-overexpressing rice with a proteasome inhibitor MG132 induced high accumulation of poly-ubiquitinated myc:WRKY45, suggesting that WRKY45 is degraded by UPS. Transcripts of WRKY45-dependent defense genes were upregulated in proportion to WRKY45 levels accumulated by MG132, suggesting a negative role of the UPS degradation in the regulation of WRKY45-dependent defense responses. Meanwhile, MG132 inhibited full upregulation of the WRKY45-dependent defense genes by SA, suggesting a positive role of the UPS degradation in the regulation of WRKY45 activity. Further analysis revealed that WRKY45 C-terminal peptide of 26 amino acids is essential for both transactivation activity and UPS degradation of WRKY45, consistent with the studies in animal and yeast proposing a close linkage of transcriptional activity and UPS degradation of transcription factors. NPR1, a central transcriptional regulator of the SA-pathway in Arabidopsis, was reported to undergo UPS degradation. However, OsNPR1, the rice counterpart of NPR1, was insensitive to MG132. Based on the data, we will discuss the roles of WRKY45 and OsNPR1 in the rice SA-pathway and significance of the UPS regulation of these SA-pathway components.

PS10-394

Functional analysis of bHLH transcriptional factors MYL1, MYL2 and MYL 3 in jasmonate signaling

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Jasmonates are plant hormones which play crucial roles in developmental processes and defense responses against wounding and pathogens. COI1 mediates jasmonate signaling by promoting the hormone-dependent degradation of JAZ proteins. JAZ proteins are repressors of MYC2, a JA responsive transcription factor. After the degradation of JAZs by SCF^{COI1}, MYC2 is thought to be released allowing it to regulate the expression of jasmonate-responsive genes. Apart from MYC2, information on other transcriptional factors that regulate jasmonate signaling is rather limited. Based on our co-expression analysis, we focused on a subset of bHLH transcription factors designated as MYL1, MYL2 and MYL3. We obtained and analyzed T-DNA insertional mutants of each MYL gene. Contrary to the *myc2* phenotype, *myl1myl2myl3* mutants were hypersensitive to methyl jasmonate (MJ). To characterize genes regulated by MYL1, MYL2 and

MYL3, we performed GeneChip analyses of *myl1myl2myl3* and Col. We observed a set of genes that showed enhanced jasmonate responsiveness in *myl1myl2myl3*. For example, *PAP1* and *MYB113*, positive regulators of anthocyanin biosynthesis, showed increased expression in *myl1myl2myl3* after MJ treatment. We confirmed that anthocyanin levels in *myl1myl2myl3* were higher than Col after MJ treatment, and therefore we conclude that *MYL1*, *MYL2* and *MYL3* negatively regulate anthocyanin biosynthesis. We also found that jasmonate metabolic genes show increased expression in MJ treated *myl1myl2myl3*. In wounded *myl1myl2myl3* leaves, the contents of jasmonic acid (JA) and JA metabolites were higher than Col. These results suggest that *MYL1*, *MYL2* and *MYL3* function as negative regulators of jasmonate metabolism.

PS10-395

MED25 integrates jasmonate associated transcription in *Arabidopsis thaliana*

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The Mediator complex functions as a universal adaptor between transcription factors and the RNA polymerase II complex to activate gene expression. In *Arabidopsis thaliana*, the MED25 subunit of the Mediator complex has been shown to be a positive regulator of jasmonate- (JA) associated gene expression as well as a regulator of flowering time, cell size and abiotic and biotic stress. Using yeast 2-hybrid and protein pull-down experiments, we show that MED25 physically interacts with several known transcriptional regulators of the JA signaling pathway, including the AP2/ERF transcription factors (TFs), ORA59 and ERF1, as well as the basic helix-loop-helix TFs MYC2, MYC3 and MYC4. Using *in planta* transcriptional activation experiments, we show that ORA59 and ERF1 require a functional MED25 to activate expression of the *PLANT DEFENSIN1.2 (PDF1.2)* gene. In addition, MED25 is also required for MYC2-dependent activation of the insect defense gene, *VEGETATIVE STORAGE PROTEIN1 (VSP1)* as well as MYC2-dependent repression of pathogen defense genes. These results suggest an important role for MED25 as a regulatory point within the Mediator complex for the control of JA-associated herbivore and pathogen defense genes.

PS10-396

Function of COI1 in *N* gene-mediated resistance to *Tobacco mosaic virus* in tobacco

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In *N* gene-containing tobacco, infection with *Tobacco mosaic virus* (TMV) triggers rapid and localized cell death at the infection site, known as a hypersensitive response, resulting in the formation of necrotic lesions that limit viral multiplication and systemic spread. We have previously shown that tobacco plants, in which the tobacco mitogen-activated protein kinases WIPK and SIPK were silenced, exhibited reduced jasmonic acid (JA) accumulation and enhanced local resistance to TMV, suggesting that JA accumulation negatively regulates local resistance to TMV. This is inconsistent with a previous study by Liu et al. (2004) using a transient expression system with *Nicotiana benthamiana*, who reported that VIGS of COI1, a central component of JA signaling, attenuates *N*-mediated resistance to TMV. However, *N. benthamiana* is known to exhibit enhanced susceptibility to infection with some viruses including TMV. To determine the exact role of JA in resistance to TMV, we used stable transgenic tobacco plants in which *COI1* was silenced. First, we examined the effect of JA on resistance to TMV. Exogenously applied methyl jasmonate (MJ), a methyl ester of JA, increased the size of local necrotic lesions and the degree of TMV accumulation compared with control treatment. Next, *COI1*-silenced tobacco plants were assayed for TMV resistance. Silencing of *COI1* reduced the size of necrotic lesions and the degree of TMV accumulation in inoculated leaves. These results suggest that COI1-dependent JA signaling negatively regulates resistance to TMV in *N* gene-containing tobacco.

PS10-397

Involvement of auxin transcriptional repressor IAA8 on the regulation of programmed cell death via direct interaction with LSD1 in *Arabidopsis*

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Plant programmed cell death (PCD), including the hypersensitive response associated with successful immune responses, results from cellular responses to oxidative stresses. *Arabidopsis* LSD1 is a negative regulator of PCD and may control the expression of cell death-related genes by altering intracellular partitioning of transcriptional regulators. IAA8, one of the Aux/IAA auxin transcriptional repressors, was identified as an interaction partner of LSD1 by yeast two-hybrid (Y2H) screening. Interestingly, IAA8 and additional Aux/IAAs interacted with LSD1 via the conserved domain II which is also required for Aux/IAA interactions with the TIR1/AFB auxin receptors. In transient protoplast assays, we found that most Aux/IAA-GFPs accumulated in the nucleus. Contrastingly, IAA8 and IAA18, both LSD1 interactors, accumulated in both the nucleus and cytoplasm. Mutations in the conserved IAA sequence required for the Y2H interaction with LSD1 compromised the cytoplasmic localization of IAA8-GFP, but not IAA18-GFP. *In planta* BiFC analyses indicated that LSD1 interferes with auxin-dependent binding of IAA8 to TIR1. We further demonstrated that *IAA8* is a negative mediator of the uncontrolled cell death observed in *lsd1*. Microarray and expression analyses revealed that a subset of auxin-responsive genes are up-regulated in *lsd1* and that up-regulation of marker genes for auxin and cell death in *lsd1* is controlled by *IAA8*. These results suggest that IAA8 may participate in the initiation of PCD by modulating transcriptional regulation on auxin signaling via direct interaction with LSD1 in the cytoplasm.

PS10-398

Rice OsERF922 negatively regulates basal resistance and salt tolerance modulated by ABA

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APETELA2/ethylene response factor (AP2/ERF) transcription factors play important roles in plant development and in the responses of plants to biotic and abiotic stresses. There are 122 members in Arabidopsis and 139 in rice. Rice *OsERF922* is strongly induced by abscisic acid (ABA) and salt treatments as well as by both virulent and avirulent pathovars of *Magnaporthe oryzae*. OsERF922 is localized to the nucleus, binds specifically to the GCC box *in vitro* and acts as a transcriptional activator in plant and yeast cells. The elevated disease resistance against *M. oryzae* of RNAi plants was associated with increased expression of *PR*, *PAL*, and phytoalexin biosynthesis related genes with and without pathogen infection. By contrast, overexpressing lines

showed reduced expression levels of these defense-related genes and enhanced susceptibility to *M. oryzae*. In addition, *OsERF922* overexpression lines became more sensitive to salt stress in rice and tobacco plants. Expression of ABA biosynthesis-related gene, nine-*cis* epoxycarotenoid dioxygenase (NCED) *4* and accumulation of ABA were decreased in the *OsERF922* RNAi plants, while increased in the overexpression lines. These results suggest that OsERF922 functions as a negative regulator of plant disease resistance and salt tolerance mediated through ABA and is integrated into the complicated cross-talk between biotic and abiotic stress signaling networks.

PS10-399

Involvement of the JA-inductive bHLH transcription factor RERJ1 in rice defense responses

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Plant hormone jasmonate (JA) is a signal molecule that is induced by various stresses such as wounding, herbivory, and pathogen attack and mediates defense reactions. RERJ1 (rice early responsive to JA 1) is a JA-inducible bHLH transcription factor, whose mRNA expression is rapidly induced in response to wounding and JA within 10 minutes. Recently, we found that RERJ1 expression was induced only at the region of injury after wounding by using RERJ1 promoter-GUS transgenic plants and suggested that RERJ1 plays a role as a transcriptional activator for regulating stress-inducible gene expression, with a strong correlation to JA accumulation in the stressed region. To gain further understanding of RERJ1 function, here we performed transcriptome analysis under wounding condition and bioassay for insect resistance using *rerj1-Tos17* mutant. Transcriptome analysis revealed that expressions of many defense related genes including proteinase inhibitor genes were greatly suppressed in wounded *rerj1* mutant. Armyworm feeding experiments showed that the weight of *rerj1*-fed larva was remarkably higher than that of wild-type-fed larva and that the area of feeding damage in *rerj1* mutant was bigger than wild-type plant. We also found that expression of the gene for the monoterpenoid linalool production was not induced obviously after wounding in *rerj1* mutant. It is known that the release of plant volatiles such as mono- and sesquiterpenoids is induced under stress conditions and affects plant-insect interactions. Taken together, these results show that RERJ1 plays a significant role in the transduction of wound signals to acquire the resistance to herbivory in rice.

PS10-400

Jasmonate signaling pathway through JASMONATE-ZIM DOMAIN (JAZ) protein in rice

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Jasmonate (JA) is a phytohormone that regulates plant development and protection against biotic and abiotic stresses. JA signaling pathway has been extensively studied in *Arabidopsis thaliana* . The JASMONATE-ZIM DOMAIN1 (AtJAZ1) protein is a transcriptional repressor of JA signaling, which binds and inhibits transcriptional factors in JA signaling. The bioactive jasmonoyl-isoleucine (JA-Ile) promotes the interaction between AtJAZ1 protein and the F-box protein CORONATINE INSENSITIVE1 (COI1), which interaction causes the degradation of AtJAZ1 protein by 26S proteasome. This degradation allows transcriptional factors, such as MYC2 and MYB21, to activate JA-responsive genes. However, JA signaling mechanisms of rice involving JAZ protein are poorly understood compered to those of *Arabidopsis* . We focused on a JAZ protein of rice (OsJAZ) and analyzed its protein-protein interaction by yeast two-hybrid system (Y2H) using coronatine (COR) as a substitute for JA-Ile. Interaction between OsJAZ and OsCOI1 required COR, however, that between OsJAZ and OsMYC or OsMYB did not. These results suggest that rice possesses JA signaling pathway mediated by JAZ protein as well as *Arabidopsis* .

PS10-401

Effect of environmental stress on induced disease resistance by plant activators and endophytic bacteria in rice

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Induced disease resistance, activated by some microbes and chemicals, protects the whole plant from the attacks by various types of pathogens such as bacteria, fungi and viruses. In this study, we analyzed changes of phytohormone signaling during the induction of these resistances. Systemic acquired resistance (SAR) is induced by pathogen infection through salicylic acid (SA) accumulation. SAR has been practically utilized in rice fields by exploiting the plant activators capable of inducing SAR. Extensive studies in Arabidopsis have revealed that the SA-mediated signal transduction for SAR induction is antagonistically regulated by the ABA-mediated signaling for environmental stress responses. However, it remains to be clarified whether SAR in rice is regulated in the same manner. On the other hand, rice plants colonized with endophytic bacteria exhibited disease resistance against rice blast and rice bacterial blight, however, its induction mechanism is still unknown. To clarify the detailed mechanisms of these resistances in rice, we investigated phytohormone signaling by analyzing phytohormone levels using LC-MS/MS and gene expression levels in the sections of leaves. The treatment of a plant activator BIT induced SA accumulation and defense related genes expression in rice leaves. However, BIT-induced SA accumulation and defense related genes expression were inhibited by environmental stress responses signaling. These results indicated that SA levels are important of SAR in rice.

PS10-402

Salicylic acid and ethylene induce resistance to *Phytophthora sojae* in soybean (*Glycine max*)

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Stem and root rot disease of soybean caused by the oomycete pathogen *Phytophthora sojae* is among the most devastating ones in soybean-producing area worldwide. Here we investigated protective effects and the molecular mechanism of various plant hormones and plant activators on soybean seedlings against *P. sojae*. We found that application of benzothiazole (BTH), an activator of the salicylic acid (SA) signaling, and 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene (ET) biosynthesis, remarkably enhanced resistance against *P. sojae* in soybean seedlings. In contrast, gibberellin (GA) and abscisic acid (ABA) rendered the soybean seedlings more susceptible to *P. sojae*. Co-treatment of ABA with BTH or ACC negated the protective effects of BTH and ACC, indicating ABA acts antagonistically on SA and ET signaling pathways. On the other hand, GA did not interfere with BTH or ACC. The BTH and ACC themselves did not inhibit fungal growth of *P. sojae* and co-treatment of ACC together with

its analog α-aminoisobutyric acid (AIB) diminished the protective effect, suggesting that ET biosynthesis was required for ACC-induced soybean resistance. Expression analysis of ET- and SA-responsive genes revealed the activation of ET and SA signaling pathways during *P. sojae* infection. In addition, ACC treatment primed expression of the ET-responsive genes and pathogenesis-related genes *PR-1* and *PR-4*. Taken together, our results suggest that ET-induced soybean resistance against *P. sojae* relies on priming of defense-related genes.

PS10-403

Development of multi-disease resistant rice by optimized overexpression of *WRKY45*

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Rice transcription factor WRKY45 plays a central role in induced disease resistance by plant activators through the salicylic acid signaling pathway. Transgenic rice plants overexpressing *WRKY45* driven by maize ubiquitin promoter (P_{ZmUbi}*:WRKY45*) exhibited strong resistance to rice blast and bacterial leaf-blight diseases, accompanying relatively minor growth defects presumably due to priming effect. However, an unknown environmental factor(s) triggered defense responses in these plants, leading to growth defects. To optimize *WRKY45* expression in regard to disease resistance and agricultural traits, we generated several transformant rice lines overexpressing *WRKY45* driven by various new constitutive promoters from rice. We isolated 2-kb upstream sequences of 22 rice genes that are expressed at various levels as estimated from microarray and database analyses, fused upstream of *WRKY45* cDNA, and transformed rice with these constructs. Among the transformants, P_{OsUbi7}*:WRKY45* lines, which express *WRKY45* driven by *OsUbi7* promoter, exhibited the best results. They exhibited enhanced resistance to both blast and leaf-blight diseases. In addition, their agricultural traits were greatly improved compared with P_{ZmUbi}*:WRKY45* lines and nearly comparable with those of control Nipponbare rice both in a greenhouse and an isolation field. Histochemical analysis using P_{OsUbi7}*:GUS* lines showed that P_{OsUbi7} has a constitutive promoter activity. In P_{OsUbi7}*:WRKY45* lines, basal expression levels of defense genes were low and the genes were rapidly induced after blast infection. These results suggest that stable primed state is established in these lines. Overall, the P_{OsUbi7}*:WRKY45* lines are optimized for disease resistance and agricultural traits, and therefore promising for practical application.

PS10-404

Chloroplast-mediated plant innate immunity through chloroplast Ca^{2+} sensor protein CAS.

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Plant immunity activates two parallel signal transduction pathways: cytoplasmic signaling pathways, such as mitogen-activated protein kinase (MAPK) phosphorylation cascades and Ca^{2+} signaling pathways, leading to transcriptional reprogramming, and a chloroplast-mediated pathway leading to the generation of chloroplast-derived reactive oxygen species (ROS) and the production of defense-related hormones, such as salicylic acid (SA) and jasmonic acid (JA). However, the molecular link between the chloroplast and cytoplasmic-nuclear immune signaling pathways remains largely unknown. Here, we show that pathogen-associated molecular pattern (PAMP) signals are quickly relayed to chloroplasts and evoke specific Ca^{2+} signatures in the stroma. We further demonstrate that a chloroplast-localized protein, CAS (calcium-sensing receptor), is involved in the regulation of stromal Ca^{2+} and responsible for both PAMP-induced defense responses and *R* gene-mediated hypersensitive cell death. CAS likely acts upstream of PAMP-induced ROS signaling and SA biosynthesis, allowing chloroplast control on plant innate immunity. Transcriptome analysis in early defense response demonstrates that CAS is involved in PAMP-induced expression of defense genes and suppression of chloroplast genes probably through $^1O_2$-mediated retrograde signaling. WRKY family transcription factors might play a critical role in chloroplast-mediated transcriptional reprogramming in plant innate immunity. This study reveals a previously unknown chloroplast-mediated signaling pathway linking chloroplasts to cytoplasmic-nuclear immune responses.

PS10-405

Dynamic changes in histone modifications during ABA-mediated suppression of defense-related genes

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Plants are always exposed to various kinds of stresses, biotic and abiotic. They have survived severe conditions by activating suitable responses to the environmental stresses using some phytohormones, such as salicylic acid (SA), abscisic acid (ABA) and jasmonic acid (JA). It is well-known that there is a strictly regulated balance between these phytohormone-induced responses under different stress conditions. Systemic acquired resistance (SAR) induced by plant activator is one of plant responses against broad pathogens via SA signaling. Previously, we showed that treatment of ABA suppressed SAR induction by inhibiting the pathway both upstream and downstream of SA in *Arabidopsis*. (Yasuda et al., 2008) However, the detailed mechanism of suppression effect of ABA is still unknown. Recently, it is reported that the regulation of the gene expressions in SA and ABA signaling pathways are closely related to the change of histone modification pattern of these genes. To elucidate the dynamic changes in chromatin structure via histone modifications in stress response and modulation of these SA-inducible genes, expressions, suppressed by ABA, we analyzed the chromatin state in *Arabidopsis* by Chromatin immunoprecipitation (ChIP) assay. We demonstrate that the suppression of SA-inducible genes by ABA treatment associated with the general histone modifications, H3K4me3 and H3K9ac. In future, we plan to try genome-wide analysis of the histone modifications by ChIP-Seq.

PS10-406

Mitochondrial complex II plays a critical role in defense against diverse pathogens

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Plant glutathione S-transferases (GSTs) are detoxifying enzymes involved in protecting tissues from oxidative damage or toxic products. One well-studied GST is GSTF8, whose gene expression can be induced by a range of elicitors including pathogen attack and signalling molecules such as salicylic acid (SA), hydrogen peroxide (H_2O_2), and auxin and its synthetic herbicide counterparts. Its early transcriptional response to these stressors has made it a commonly used marker gene for early stress and defense responses. To gain

insight into mechanisms of early stress responses we conducted a forward genetic screen to identify mutants with changes in *GSTF8* promoter activity. One such mutant, disrupted in stress response (*dsr1*), showed loss of stress inducible *GSTF8* expression, altered SA-regulated gene expression, and increased susceptibility to specific pathogens including the bacterial and fungal pathogens *Pseudomonas syringae* and *Rhizoctonia solani* respectively. We mapped the *dsr1* mutation to a single amino acid change in the mitochondrial complex II succinate dehyrogenase subunit SDH1-1 and show reduced mitochondrial produced reactive oxygen species (ROS) within this mutant. While ROS play major roles in response to pathogen attack, little is known about the role of localised mitochondrial derived ROS. Our identification of *dsr1* provides genetic proof that mitochondrial generation of ROS plays a critical role in plant stress and defense responses. We have subsequently characterised further *GSTF8* promoter mutants with altered defense gene expression and some exhibiting increased pathogen resistance. These mutants are proving to be a valuable resource into expanding our understanding of early plant stress responses.

PS10-407

Blast resistance by panicle blast resistant gene *Pb1* is mediated by WRKY45

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Panicle Blast1 (*Pb1*) is a panicle blast resistance gene derived from *indica* rice "Modan". *Pb1* encodes a CC-NB-LRR protein and confers rice with durable resistance of broad spectrum for fungal races. Here, we investigated molecular mechanisms underlying *Pb1*-mediated blast resistance. Pb1 protein interacted with a transcription factor WRKY45, which plays a central role in induced resistance via the salicylic acid signaling pathway and is regulated by ubiquitin proteasome system (UPS). To test the WRKY45 dependence of *Pb1*-mediated blast resistance, we knocked down WRKY45 in a *Pb1*-containing cultivar and *Pb1*-overexpressed (*Pb1*-ox) Nipponbare. In both cases, *Pb1*-mediated blast resistance was largely compromised by *WRKY45* knockdown, suggesting that *Pb1* resistance is dependent on WRKY45. Overexpression of *Pb1-Quad*, a CC-domain mutant that interacts with WRKY45 very weakly, resulted in markedly weaker blast resistance than that of wild-type *Pb1*, indicating that the interaction with WRKY45 through the CC domain is required for *Pb1*-mediated blast resistance. Overexpression of *Pb1* with nuclear export sequence in rice failed to confer blast resistance, indicating that nuclear localization is necessary for Pb1 function. In a transient system using rice cultured cells, co-expression of Pb1 with WRKY45 enhanced accumulation of WRKY45 proteins and increased WRKY45-dependent transactivation activity. Blast infection induced increased accumulation of WRKY45 in *Pb1*-ox than in control Nipponbare. These results suggest that *Pb1* resistance is mediated by protection of WRKY45 from its UPS degradation.

PS10-408

Identification of naringenin 7-*O*-methyltransferase, a key enzyme in the biosynthesis of the flavonoid phytoalexin sakuranetin in rice

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Sakuranetin is the only flavonoid phytoalexin in rice, whose production is induced by ultraviolet (UV) irradiation, $CuCl_2$ or jasmonic acid (JA) treatment, and pathogen infection. Recent studies suggest the usefulness of sakuranetin not only as a plant antibiotic compound but also as a potential pharmaceutical agent exhibiting various bioactivities. A naringenin 7-*O*-methyltransferase (NOMT) is known to catalyze the final step of sakuranetin biosynthesis. Previous attempt to isolate rice NOMT (OsNOMT) from UV-treated wild-type rice leaves was unsuccessful and a caffeic acid *O*-methyltransferase, OsCOMT1, was identified instead. In this study, we demonstrated that OsCOMT1 does not contribute to sakuranetin production in rice *in vivo*, and we successfully purified OsNOMT using the *oscomt1* mutant. A crude protein preparation from UV-treated *oscomt1* leaves was subjected to three sequential purification steps, resulting in a 400-fold purification from the crude enzyme preparation. The purified fraction contained protein with an apparent molecular mass of 40 kDa. MALDI-TOF/TOF analysis and subsequent database searches enable us to identify the genes for two *O*-methyltransferase-like proteins from the 40 kDa band. Since one of the recombinant proteins encoded by *Os12g0240900* gene showed NOMT activity with reasonable kinetic properties, we concluded that *Os12g0240900* gene encodes an OsNOMT. The expression of *OsNOMT* gene was transiently induced by JA treatment in rice leaves prior to sakuranetin accumulation. We are now generating transgenic rice plants modifying the *OsNOMT* gene expression to investigate how the changes of sakuranetin level affect blast-resistance in rice.

PS10-409

The AP2/ERF domain transcription factor ERF104 confers resistance to necrotrophic fungi via salicylate and ethylene signaling pathways, but not jasmonate pathway

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Pathogenic infection of plants induces a variety of defense responses that depend on action of endogenously produced hormones, including salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). In this study, we identified a novel gene, ETHYLENE-RESPONSE-FACTOR104 (ERF104) from Brassica napus and Arabidopsis thaliana. AtERF104 expression was suppressed by ET and in the mutant npr1-1, induced by SA and in the mutant ein2-1, and independent of JA and not affected in the mutant coil-1. Over-expression of AtERF104 whose protein specifically binded to the GCC-box cis element activates expression of several pathogenesis-related (PR) genes, including ET-responsive genes PDF1.2 and ChiB, and SA-responsive genes *PR-1* and *PR-2* while expression of these genes except for *PR-1* was down-regulated in *AtERF104*-silencing plants. Consistently, *AtERF104* over-expression increased plant resistance against necrotrophic fungi Botrys cinerea and Sclerotinia sclerotiorum while *AtERF56*-silencing plants decreased resistance to both pathogens. Our results suggested that ERF104 functions as a negative regulator in the ET signaling pathway and as a positive regulator in the SA signaling pathway, independent of the JA pathway, in plant defense responses against necrotrophic fungi.

PS10-410

Role of JAZ protein in jasmonic acid-induced resistance to rice bacterial blight in rice

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Jasmonic acid (JA) is a natural hormone involved in development, responses against wounding, pathogen and insect attack. The JA biosynthetic pathway has been well studied, and much information about the type and subcellular localization of its enzymes is available in many plant species. And the few signaling components have been identified by *Arabidopsis* mutant screens displaying a reduced sensitivity to JA. In contrast, information about the JA signaling

pathway in rice (*Oryza sativa* L.) is limited. As a first step toward understanding the role of JA signaling in rice disease resistance, we investigated effect of JA against rice bacterial blight disease which caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in rice, one of the most serious rice-plant disease in rice growing countries. Rice plants showed increased resistance against *Xoo* by JA treatment. A large-scale screening using a rice DNA microarray revealed that many defense-related genes involved in rice resistant response were upregulated by JA. We next analyzed the role of JAZ protein using JA-insensitive transgenic rice plants overexpressing JAZ protein truncated with C-terminal region. As a result, expression of many defense-related genes upregulated by JA was suppressed in transgenic rice plants. Furthermore, JA-induced resistance against *Xoo* was cancelled in the JA-insensitive transgenic rice plants. Based on these data, we conclude that upregulation of defense-related genes by JA was an important response in rice resistant response against *Xoo* and was regulated by JAZ protein in rice.

PS10-411

Role of jasmonic acid-induced volatile in resistance to rice bacterial blight in rice

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Jasmonic acid (JA) is one of the plant hormone involved in response to pathogen and wounding. There are few information about JA signaling pathway in disease resistance in rice. We have demonstrated that JA signaling plays an important role on resistance to rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In addition, we found that many volatiles were accumulated by JA treatment in rice. Thus, we investigated whether JA-induced volatiles play a role on resistance to *Xoo* in rice. Among JA-induced volatiles, one monoterpene compound, linalool, was reproducibly accumulated by JA treatment in rice. It has been reported that some monoterpenes have antibacterial activity to plant bacterial pathogens. Therefore, we first analyzed the antibacterial activity of linalool to *Xoo*. As a result, a direct treatment of linalool to liquid culture of *Xoo* had no effect on the growth of *Xoo*, indicating that linalool has no antibacterial activity to *Xoo*. However, we found that a vapor treatment of linalool to rice induced resistance to *Xoo*. Vapor treatment with linalool caused upregulation of gene expression involved in defense response in rice. We next produced transgenic rice plants accumulating linalool by overexpressing linalool synthase gene. These transgenic rice plants showed highly expression of defense-related genes under normal condition without any treatment. Furthermore, these plants showed increased resistance to *Xoo*. Based on these date, we conclude that linalool plays an important role on resistance to *Xoo* in rice.

PS10-412

Plasmodesmata and defense signaling: mechanisms and players

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Cell-to-cell communication mediated by plasmodesmal channels is thought to play fundamental roles not only in physiological and developmental processes but also during interactions with pathogens. However, how the spatiotemporal changes in plasmodesmal permeability are controlled in response to microbial infection and how this this regulation impacts overall innate immunity of the plant are not well understood. Recently, we have found that the crosstalk between salicylic acid (SA)-mediated defense signaling and the control over plasmodesmata-mediated cell-to-cell communication is crucial for innate immune responses against bacterial pathogens (Lee et al., Plant Cell 2011; 23:3353-73). This crosstalk is facilitated by a plasmodesmata-located membrane protein named PDLP5, which functions as both negative and positive regulator of plasmodesmata and SA signaling pathway, respectively. To gain further insight into this novel discovery, we performed comprehensive cellular and genetic analyses by employing a combination of tools we have developed to assay plasmodesmal permeability in real time and Arabidopsis mutants that are impaired in SA biosynthesis/ signaling in the background of PDLP5 mutants. Here we present detailed mechanisms by which the defense hormone SA in response to bacterial infection regulates plasmodesmal closure and structural modification. Furthermore, genetic players involved in this process and the epistatic relationships between the identified players and PDLP5 will be presented. We will also discuss how this integration of the defense pathway and plasmodesmal connectivity might contribute to the balancing between the controlled cell death and survival of infected plants.

PS10-413

The interplay between cytokinin and salicylic acid signaling coordinates activation of defense responses in Arabidopsis

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The past decades have revealed an important role for hormones in plant immunity. We are now beginning to understand the contribution of crosstalk among different hormone signaling networks to the outcome of plant-pathogen interactions. Cytokinins are plant hormones involved in the regulation of many aspects of plant development and responses to the environment. In *Arabidopsis*, cytokinin signaling involves a phosphorelay pathway similar to two-component response systems used by bacteria and yeast to perceive and react to various environmental stimuli. In previous studies (Argueso et al., PLoS Genetics 2011) we have uncovered that components of cytokinin signaling contribute to limiting the growth of a pathogenic isolate of the oomycete *Hyaloperonospora arabidopsidis* in Arabidopsis plants, through the interplay between the plant hormones cytokinin and salicylic acid (SA). Pre-treatment of *Arabidopsis* and rice plants with cytokinin leads to enhancement of SA-dependent defense responses upon pathogen exposure, in a process similar to defense priming. These functions for cytokinin in plant immunity require an intact host cytokinin phosphorelay system, and are mediated in part by type-A ARRs, which act as negative regulators of defense gene expression. We are now continuing to explore the role of cytokinins in plant immunity and defense priming with a focus on type-A ARR function.

PS11-414

Characterising the genetic basis of *E. coli* O157:H7 survival in the plant environment

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The circulation of human bacterial pathogens within the food chain is becoming more and more apparent, presenting itself as a rapidly growing threat to food safety. *Escherichia coli* O157:H7 (O157) is often harboured within the intestines of healthy cattle and high densities are shed asymptomatically in their faeces. Animal manure is increasingly being used as a plant fertilizer where O157 can come into intimate contact with plants, become internalized within certain plant tissue and spread systemically via the vascular system. Consumption of infected produce may then well lead to infection within the human host. Plant colonization and

growth dynamics of O157 have been investigated in pea seedlings. O157 has been demonstrated to survive for up to 3 weeks. An *in silico* comparative approach coupled with promoter-probe *in vivo* expression technology (IVET) screen has been used to identify candidate genes up-regulated during infection and therefore likely to be important for plant colonisation. Mutational analysis and fitness assays have been undertaken to assess the importance of specific genes for their role in plant colonisation. Experimental evolution of O157 through passaging of the pathogen has revealed several clones which seem able to survive for extended periods of time as compared to the wildtype. Electron microscopy has shown several distinct morphological differences between these. Genome re-sequencing and annotation should reveal interesting information on possible point mutations. Taken together, this project aims to better understand how O157 survives in the environment and provide insight on how to prevent human infections.

PS11-415

Cloning, characterization and expression of an insecticidal crystal protein gene from *Bacilllus thuringiensis* isolates of Andaman and Nicobar Islands

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The six isolates of *Bacillus thuringiensis* from Andaman and Nicobar Islands which were previously characterized by PCR analysis for the presence of Coleopteran active *cry* genes were used for Cry1I full length gene amplification. A 2.16-kb DNA fragment of Cry1I gene was PCR amplified, cloned in expression vector pQE 80 L, and then used for transformation of *E. coli* M15 cells. The optimum expression was obtained with 1 mM IPTG at 37°C for 3 h. The sequence of the cloned crystal protein gene showed almost complete homology with a Cry1I toxin gene from *Bacillus thuringiensis* var. *kurstaki*, with scattered mutations in the toxic region. The deduced sequence of the protein has homologies of 91.0% with Cry1I and Cry1Ia, and 98.0% with Cry1Ib. Cloning of this gene may help to overcome the increasing resistance of pests to currently used insecticides. Based on the results obtained, the PCR method may be a valuable and reliable tool for specific detection and identification of *cry1I* genes. The toxicity of Bt recombinant protein was determined against first instar larvae of *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) and Adults; *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) at 310 µg/mL and 15.5 µg/mL, respectively. The novel *cry1I* gene will be an important resource in constructing genetically engineered bacteria and transgenic plants for biocontrol of insect pests and Bt based biopesticidal formulations, aiming to reduce the use of chemical insecticides.

PS11-416

Elevation of soil microbial enzyme activities and reduction of fusarium wilt disease incidence by chitin amendment in tomato field plots

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The effect of chitin amendment on soil microbial enzyme activities and fusarium wilt disease were evaluated in tomato planting field plots. Soil analyses were performed on samples taken periodically at one week before transplanting, four weeks after transplanting and one week after harvesting from each 1 x 6 meter experimental plot. Significant improvement of the selected soil parameters were achieved in both 500 g and 1,000 g amended plots. Chitinase activities of the 500 g and 1,000 g amended plots were elevated to 1.81 and 2.86 folds at four weeks after transplanting and 1.33 and 1.81 folds at one week after harvesting. Similarly, the dehydrogenase activities were increased up to 3.65, 4.47 and 1.63, 1.64 folds at respective conditions. While the increasing rates of chitinase and dehydrogenase activities in the un-amended plots were 1.47, 1.03 and 1.32, 1.23 folds, respectively. Determination of total microbial activity by fluorescein diacetate hydrolysis was also carried out to explore an organic matter turnover. Similar elevation pattern was gained with the 500 g chitin, 1,000 g chitin amended and un-amended plots up to 1.73, 2.12 and 1.23 folds at four weeks after transplanting and 1.19, 1.72 and 1.01 folds at one week after harvesting, respectively. Meanwhile the reduction of fusarium wilt disease incidence as much as 52% to 62% were achieved by the amendment of 500 g and 1,000 g chitin into tomato planting- soil plots. These results are thus markedly accountable for the sustainable benefits of a biomaterial chitin.

PS11-417

Use of essential oils for the control of post harvest decay in citrus

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The present study was designed to evaluate (*in vitro* and *in vivo*) antifungal activities of the essential oils obtained from Cumin seeds, Clove buds and Cinnamon bark against *Penicillium italicum* that is the causal agent of blue mold disease in citrus fruit during storage. Different concentrations (3, 6, 12, 24 and 48 µl/mL) of selected essential oils were checked for their potential to inhibit the mycelial growth of the test fungi. Overall various assays confirmed the potential of tested essential oils for their antifungal activity which varied with type and concentration of oil used. The in vitro study revealed that the essential oils of cumin and clove have the potential to inhibit mycelial growth of test fungi completely at concentrations of 12 and 48 µl/ml respectively. Essential oil of cinnamon, however failed to completely inhibit the mycelial growth even at maximum used concentration of 48 µl/ml. *In vivo* assays also support these outcomes. Clove and cumin oils when applied on citrus fruits, showed total fungal inhibition at concentration of 24 µl/ml and 48 µl/ml respectively. Whereas, cinnamon essential oil could not prevent fungal infection even when used in highest tested concentration. The study was extended to the identification of active components of the three oils. Clove oil shows the presence of eugenol, alpha-terpineol, Isoeugenol and beta-terpinene as its major components. The chief components found in cinnamon oil were eugenol and cinnamaldehyde, whereas cumin oil revealed the presence of gamma-terpinen, cuminaldehyde and 4-carvomenthenol.

PS11-418

Imprimatins, novel plant immume-priming compounds identified *via* a newly-established high-throughput chemical screening target salicylic-acid glucosyltransferases in *Arabidoposis thaliana*

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Plant activators are compounds that protect plants from pathogens

by activating their immune system. Compared with commonly-used pesticides which target pathogens, plant activators provide durable effect to broad spectrum of diseases which have not been overcome by pathogenic microbes. Although several plant activators such as probenazole and benzothiadiazole have been widely used in agriculture, the molecular mechanisms of immune induction are largely unknown. Here we report the establishment of a high-throughput chemical screening procedure to identify plant immune-priming compounds which potentiate but do not directly induce cell death in *Arabidopsis* cell suspension cultures induced by *Pseudomonas syringae* pv. *tomato* DC3000 *avrRpm1*. From screening of a commercial library of 10,000 structurally diversified small organic molecules and derivative analysis of the isolated candidates, we identified five compounds designated Imprimatins for "immune priming chemicals". These compounds were classified into two groups, ImprimatinA and -B, with structural similarity. These Imprimatins enhanced disease resistance against both virulent and avirulent *Pseudomonas* bacteria in *Arabidopsis* plants. Pretreatments increased the accumulation of endogenous salicylic acid (SA), but reduced its metabolite, SA-O-β-D-glucoside. We found that inducing compounds inhibited both a known and a previously unknown SA glucosyltransferase (SAGT) *in vitro* in a competitive manner with SA. Each single and their double knockout *Arabidopsis* plants for these SAGTs phenocopied the Imprimatin-induced phenotypes and exhibited enhanced disease resistance. Our results indicate that Imprimatins can provide a novel mode of action to prime plant immunity, and that SA glucosylation is a target for developing novel crop protectants.

Poster 11

PS11-419

Effect of monoterpene, sabinene, to rice pathogens

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Unlike most major plant species, citrus plants contain large volumes of essential oils that are mainly composed of monoterpenes. Monoterpenes are one of the universal volatile components of plants and are synthesized by various types of monoterpene synthases from geranyl pyrophosphate in chloroplast. It has been also suggested that monoterpenes play a role in plant defenses against herbivores and plant pathogens, and as attractants for pollinators. We have previously isolated monoterpene synthase gene *RlemTPS2*, which produces sabinene, from rough lemon (*Citrus jambhili*). In addition, our previous studies have shown that sabinene has antifungal activity against citrus fungal pathogen *A. alternata*. Generally, it is known that some of monoterpenes have a wide antifungal and antibacterial spectrum against plant pathogens. To evaluate whether sabinene has antimicrobial activity against other plant pathogens, we investigated effect of sabinene toward rice fungal pathogen, *Magnaporthe grisea*, and rice bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which cause rice blast and rice bacterial blight disease, respectively. These are serious rice diseases in rice growing countries including Japan. Sabinene had antifungal and antibacterial activity toward *M. grisea* and *Xoo*, respectively. Furthermore, a vapor treatment of rice plants with sabinene induced resistance to bacterial blight caused by *Xoo*. From these results, it was concluded that sabinene has potency as a useful natural agent for suppression rice diseases.

PS11-420

Exploiting the priming ability of *Thellungiella salsuginea* to improve biotic and abiotic stress tolerance

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Crop plants are exposed to multiple stresses including water deficit, untimely frost and pathogens throughout the growing season. To find novel traits to improve crop tolerance we study the extremophile *Thellungiella salsuginea*, which is exposed to multiple stresses in the saline subarctic regions of Yukon Territory in Canada as well as in Shandong, China. Exposure to a mild period of drought (priming stimulus) conferred greater tolerance to a second episode of severe drought in Yukon *Thellungiella*, but not in the Shandong ecotype. We hypothesized that priming by an abiotic stimulus may also lead to enhanced disease resistance in this species. Therefore we adapted the *Arabidopsis -Pseudomonas syringae* pv. *tomato* (*Pst*) pathosystem for *Thellungiella* and discovered that Shandong plants were 20-fold more resistant to *Pst* than Yukon plants and 200-fold more resistant than *Arabidopsis*. Additionally, an initial exposure to drought or salt primed Yukon *Thellungiella* for enhanced resistance to *Pst*, suggesting that an abiotic stress can prime for enhanced disease resistance in this species. To gain insight into stress tolerance and priming in *Thellungiella*, we used RNA-Seq to obtain transcriptomes of plants grown in the Yukon or in growth cabinets. A number of Systemic Acquired Resistance-associated priming genes, the FLS2 flagellin receptor, and many Resistance receptors were upregulated in Shandong compared to Yukon *Thellungiella*, suggesting that Shandong *Thellungiella* exists in a primed state. Our priming and transcriptome studies indicate that both Yukon and Shandong *Thellungiella* are reservoirs of essential biotic and abiotic stress tolerance genes.

PS11-421

Suppression of cucumber diseases by using the spent mushroom substrate of *Lyophyllum decastes* and *Pleurotus eryngii*

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The protective effect of autoclaved water extract from spent mushroom substrate (AWESMS) and autoclaved spent mushroom substrate (ASMS) of the edible mushrooms *Lyophyllum decastes* and *Pleurotus eryngii* against fungal and bacterial diseases was investigated on cucumber plants. Plants were treated with AWESMS of *L. decastes* or *P. eryngii* by dipping or spraying the first true leaf, and inoculated with the target pathogen after 1 week. Results showed that AWESMS of *L. decastes* significantly reduced diseases by *Colletotrichum orbiculare*, *Podosphaera xanthii*, and *Pseudomonas syringae* pv. *lachrymans*, but not diseases by *Corynespora cassiicola* and *Cladosporium cucumerinum*. The AWESMS of *L. decastes* showed no antifungal activity against *C. orbiculare* and a significant increase of expression of chitinase and β-1,3-glucanase genes 24 h after pathogen inoculation was observed in plants treated with the water-extract of *L. decastes*. On the other hand, when the plants were grown in a mixture (1:2, v/v) of ASMS of *L. decastes* and soil, a significant disease reduction was observed on *P. xanthii*, *C. cucumerinum* and *P. syringae* pv. *lachrymans*. Protective effect was also observed against *C. orbiculare* on plants treated with AWESMS or on plants grown in a mixture of ASMS of *P. eryngii* (1:3, v/v). Our results indicated that AWESMS and ASMS, independently of the mushroom type, provide a protective effect on fungal and bacterial diseases. Therefore, SMS should be considered an easily available source of active compounds to protect plants from fungal and bacteria infections.

PS11-422

Studies on potential roles of sulfur compounds for diseases control in the oriental pear orchard

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In order to effectively control pear diseases in the orchard, we must control primary infections. In most cases, sulfur compounds have been applied for curative and preventive purposes in the fields. Here we introduce the preventive efficacy of sulfur compounds for pear diseases control through an *in vitro* study. The object of disease control using sulfur compounds is to reduce primary inoculum on spring season. The fallen leaves from pear orchard infected by scab and other diseases were collected and treated with sulfur compounds or water as a control. Pear fruits on water-treated fallen leaves became severely infected, while they on sulfur-treated fallen leaves were fresh and uninfected after 10 days incubation. To characterize the diversity of our samples, we analyzed 18S rRNA internal transcribed spacer (ITS) regions of extracted genomic DNA from sulfur-treated or water-treated leaves, respectively. We finally identified about 20 genetically distinct fungal species. *Ascomycota* and *Cladosporium* were most common fungal species in both treated leaves. However, the population dynamics of several fungal species including *Ascomycota* and *Cladosporium* were a quite different in the each treated leaves. These results suggest that the sulfur compounds treatment led to changes of fungal communities in the oriental pear orchard.

PS11-423

Screening of metabolites derived from soil microorganisms for induction of plant resistance against *Tobacco mosaic virus*

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Soil microorganisms produce a large array of metabolites with broad bioactivity, some of which are potential disease control agents for their anti-phytopathogenic ability. Previous studies have shown that metabolites of soil microorganisms effectively reduced plant fungal and bacterial disease symptoms. However, the application of soil microbial metabolites on viral disease management remains largely to be explored. To address this issue, we utilized *Tobacco mosaic virus* (TMV) and its local lesion host, *Nicotiana glutinosa*, as a model system and established symptom quantification methods to screen soil microbial metabolites selected by a vegetable broth enhanced isolation method described by Ko et al. (2010) for anti-viral activity. Three different classes of soil microorganisms (fungi, actinomycetes, and bacteria) were selected and the initial screening results showed that two actinomycetes exhibit anti-viral activity against TMV.

PS11-424

Ethylene Response Factor (ERF) transcription factors of the B-3 subgroup include master regulators of ethylene signaling and mediate resistance to root pathogens without adversely affecting rhizobial symbiosis

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Despite being transcriptionaly induced at late time points after ethylene or pathogen application, the B-3 sub-group ERF transcription factor, AtERF14, plays a master regulatory role that is required for ethylene- and pathogen-responsive expression of other ERF genes and defence genes such as *PDF1.2*. Knock out lines displayed enhanced susceptibility to the root-infecting *Fusarium oxysporum*. In order to translate these findings for enhanced disease resistance in legume crops, homologs of *AtERF14* were studied in the model legume *Medicago truncatula*. One pathogen of importance for legume production is the root infecting fungus, *Rhizoctonia solani*. Despite extensive germplasm screens in many crops, no strong genetic resistance has been identified, suggesting alternative strategies to improve resistance in crops are required. Transcriptional analysis of Medicago revealed the specific induction of B-3 subgroup ERFs was associated with moderate resistance to *R. solani*. Over-expression of B-3 ERFs in Medicago roots increased resistance to *R. solani* as well as the oomycete, *Phytophthora medicaginis*, but not to root knot nematode. These results indicate that targeting specific regulators of ethylene defence may enhance resistance to an important subset of root pathogens. Moreover, over-expression of B-3 ERFs enhanced disease resistance without apparent impact on symbiotic interactions with rhizobium in Medicago genotype A17, while over-expression in skl reduced the hypernodulation phenotype. This suggests that under normal regulation of nodulation, enhanced resistance to root diseases can be uncoupled from symbiotic plant-microbe interactions in the same tissue and ethylene/ERF regulation of nodule number is distinct from the defenses regulated by B-3 ERFs.

PS11-425

Broad-spectrum disease resistance by *BSR1* shares transcriptional components with BTH-inducible resistance in rice

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We previously identified the rice *BROAD-SPECTRUM RESISTANCE1* (*BSR1*) gene that encodes a receptor-like cytoplasmic kinase similar to *Arabidopsis* BIK1 by using FOX hunting system. Overexpression of *BSR1* conferred resistance to *Pseudomonas syringae* and *Colletotrichum higginsianum* in *Arabidopsis*, to *P. syringae* in tomato, and to *Xanthomonas oryzae* and *Magnaporthe oryzae* in rice. To examine the function of *BSR1* in defense signaling in rice, we performed microarray analyses. Transcript levels of 642 genes were >2 fold higher in *BSR1*:OX rice compared with WT under normal growth condition. Part of these genes (17%) overlapped with BTH (SA analog)-inducible genes. On the other hand, ~40% of the BTH-inducible genes including several *WRKY* genes were upregulated in *BSR1*:OX rice. *WRKY45* is known to play a crucial role in BTH-induced blast resistance through the SA pathway and its overexpression confers resistance to both *X. oryzae* and *M. oryzae*. Microarray data suggest that broad-spectrum disease resistance in *BSR1*:OX rice is partly mediated by activation of the SA signaling including upregulation of *WRKY45*. To test this, *BSR1*:OX rice was crossed to transgenic rice overexpressing bacterial *NahG* gene (*NahG* rice) that contains undetectably low levels of endogenous SA. Transcript levels of *WRKY45* in *BSR1*:OX/*NahG* F1 plants were comparable with those in *BSR1*:OX plants and higher, while those in *NahG* rice were lower, in comparison to WT. These results indicate that the upregulation of *WRKY45* in *BSR1*:OX rice is independent of SA. Currently, studies by using loss-of-function mutants of *BSR1* are in progress.

PS11-426

Determination of R gene specificity in bread wheat

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Plants protect themselves against pathogens through a range of resistance genes (R genes). One major R gene family is composed of the nucleotide binding (NB) leucine rich repeat (LRR) type.

When a p-loop mutated NB-LRR type R gene of *A. thaliana* is introduced in a wildtype background it acts dominant negatively, conferring susceptibility to the pathogen normally obstructed by the wildtype R gene. Based on this, a strategy has been designed to match novel R gene candidates in bread wheat (*T. aestivum*) to specific pathogens. The wheat - yellow rust (*P. striiformis*) system will be used for proof-of-concept, in that the R gene *Yr10* holding the p-loop mutation will be transformed into the wheat cultivar Avocet-Yr10 harboring the wildtype gene. Transgenic lines expressing the mutant are expected to exhibit susceptibility to an appropriate avirulent *P. striiformis* isolate harboring the *AvrYr10* gene. For the subsequent screening strategy the cultivar Bobwhite S-26 will be used and a specific library of expressed NB-LRR type R gene candidates will be established based on deep transcriptome sequencing. Transgenic Bobwhite S-26 lines expressing mutated forms of R gene candidates will be inoculated with a selection of avirulent isolates of *P. striiformis*. Susceptibility to a particular isolate will reveal the specificity of the wildtype R gene corresponding to the mutant in question. The identified R gene can then be exploited in crop breeding for disease resistance.

PS11-427

Visualisation of phylloplane biofilms using Episcopic Differential Interference Contrast (EDIC) microscopy and the investigation of nitric oxide for biofilm control at the spinach phylloplane

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The control of biofilm and zoonotic pathogen colonisation at the surface of fresh produce has important implications for crop yield, food quality, and food safety. Using the spinach phylloplane as our model, we have used Episcopic Differential Interference Contrast microscopy (EDIC) coupled with Epifluorescence (EF) for the visualisation of natural phylloplane biofilms, and have introduced GFP labelled *Salmonella* for the study of human pathogenic bacterial interactions with the leaf surface. In addition, the use of the signalling molecule nitric oxide, has been investigated for its ability to influence biofilm dispersal and pathogen removal from the phylloplane. The minimal sample preparation required for EDIC microscopy allows the visualisation of leaf associated biofilm communities in their natural, unaltered state. Staining of the biofilm matrix shows they are microbial in origin, and the addition of GFP labelled *Salmonella* to leaves shows the ability of pathogens to exploit diverse environmental niches for survival at the phylloplane. The investigation of nitric oxide as a universal signalling molecule for the reduction of biofilm and pathogen contamination has shown some promising results. Understanding the complex microbial communities found in the crop production environment is essential for exploiting innate microbial behaviours, such as the nitric oxide signalling response, for our own gain. It is hoped that these studies can be applied in a crop production environment to reduce microbial spoilage and pathogen contamination of fresh produce.

PS11-428

Physiological and enzymatic characterization of *Burkholderia* spp. isoloted from cadmium contaminated soil

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Cadmium (Cd) can be added to soil through fertilizers, calcareous, pesticides and industrial and domestic effluents. It can be leached to groundwater, as well as be taken up by plants, causing damage to the environment and to human being. Adult coffee plants were cultivated in a soil without and with Cd (1.28 mM), from which tolerant bacteria from both soils with potential to be used to improve phytoremediation of contaminated soils were isolated. Two isolates of *Burkholderia* genus tolerant to different metals (5 mM of Cd, 4 mM of Ni, 15 mM Zn and 1 mM Al) were selected. The aim of this study was to verify the potential that these two isolates has to be used associated with plants to remediate contaminated soil, characterizing these two isolates enzymatically (esterase, lipase, cellulose, amylase and pectinase) and physiologically (phosphor solubilization, siderophore production and cadmium bioaccumulation). It was observed that these two *Burkholderia* isolate did not produce cellulose, amylase and pectinase enzymes, however it presented esterase and lipase enzyme activities, promoted phosphate solubilization, exhibited a high production of siderophore, which is responsible for iron chelation, but also described as cadmium chelator, decreasing its availability and toxicity to plants. The most important result was the bioaccumulation levels exhibited: 53 to 130 µg of cadmium in 100 mg of bacteria dry mass in both isolates. Data obtained for phosphor solubilization, siderophore production and Cd bioaccumulation revealed the potential of the *Burkholderia* spp isolates to be used in phytorremediation of contaminated soils.

PS12-429

Identification of a genetic factor determining the durability of a plant major resistance gene and quantitative resistance to virus accumulation

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Genetic resistance provides efficient control of crop diseases but is limited by pathogen counteradaptation. The durability of the *pvr2*^{3} allele, conferring resistance to *Potato virus Y* (PVY), was demonstrated to depend on the plant genetic background. In order to identify genetic factors affecting the durability of the *pvr2*^{3} resistance, QTL mapping was performed using doubled-haploid (DH) lines issued from the F1 between two *Capsicum annuum* lines: "Perennial" carrying *pvr2*^{3} in a partially resistant background and "Yolo Wonder" carrying the susceptible *pvr2*$^{+}$ allele in a susceptible background. 350 DH lines were genotyped with 234 markers and the linkage map was established. The 156 DH lines carrying the *pvr2*^{3} allele but segregating for the genetic background were evaluated for two traits: the breakdown frequency of *pvr2*^{3} (following inoculation with a PVY clone nonpathogenic (avirulent) towards *pvr2*^{3}) and the PVY accumulation (following inoculation with a mutant of the previous PVY clone carrying a single mutation conferring pathogenicity towards *pvr2*^{3}). Genotypic variance was highly significant for the two traits with heritabilities of 0.76 and 0.47. One major QTL, explaining 29% of the variance of *pvr2*^{3} breakdown frequency was identified on chromosome 3 and two QTLs, explaining 25% and 9% of PVY accumulation variation, were identified on chromosomes 3 and 6, respectively. Interestingly, the major QTL for the 2 traits mapped to the same region of chromosome 3. A putative pleiotropic effect affecting simultaneously the two traits, the underlying mechanism and the perspective in breeding for resistance durability will be discussed.

PS12-430

Hexose oxidase provides red algae with a mechanism for attacking bacteria

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Marine algae are believed to have their own defense strategy against pathogen infection. However, the defense systems against

pathogen infection on marine algae remain to be resolved and little is known about whether marine algae share defense mechanisms with land higher plants. Here we provide a possible mechanism underlying alga immunity, which involves in hexose oxidase (HOX)-dependent production of hydrogen peroxide (H_2O_2). We screened red algae and found that *Ptiropbora subcostata* had an ability to suppress bacterial colonization. We partially purified an enzyme contributing to the antibacterial activity in *P. sbucostata*, which has 50 % homology with HOX of *Chondrus crispus*. In-gel activity assay revealed that *P. sucostata* has an HOX activity in a hexose-dependent manner and the resistance to bacteria was completely inhibited with catalase. Furthermore, the colonization of *Bacillus subtilis* was strongly suppressed by around the alga frond of *P. subcostata* on GYP agar plate, when alga frond was placed on the plate that had been spread with spores of *B. subtilis*. These results suggest that H_2O_2 production is responsible for the suppression of bacterial colonization. Thus, our results suggest that HOX-mediated H_2O_2 production is important for marine algae to resist against bacterial pathogen in marine environment.

PS12-431

Identification and molecular mapping of a wheat gene for resistance to a *Polypogon* isolate of *Colletotrichum cereale*

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To elucidate genetic mechanisms of host species specificity in plant-microbe interactions, we analyzed interactions between anthracnose fungi and gramineous plants. Infection assays revealed that a *Sorgham* isolate (*Colletotrichum sublineolum*), an *Avena* isolate (*C. cereale*), a *Polypogon* isolate (*C. cereale*), and a *Digitaria* isolate (*C. hanaui*) were specifically virulent on the plants from which they were isolated. When 24 wheat cultivars/accessions were inoculated with a *Polypogon* isolate Cgp29, however, we found an exception; most cultivars were resistant to Cgp29 while cultivar Hope was susceptible. In F_2 populations derived from crosses between three resistant cultivars, Norin4 (N4), Chinese Spring (CS) and Shin-chunaga (Sch), and the susceptible cultivar Hope, resistant and susceptible seedlings segregated in a 3:1 ratio, suggesting that a major gene is involved in the resistance of each cultivar to Cgp29. In F_2 populations derived from crosses between the three resistant cultivars, all seedlings were resistant, suggesting that these three cultivars carry the same gene. This resistance gene was tentatively designated as *Rcg1*. Analysis with the CS-Hope chromosome substitution lines and molecular mapping revealed that *Rcg1* was located on the long arm of chromosome 5A. Cytologically, *Rcg1* was mainly associated with hypersensitive reaction. These results suggest that the resistance of wheat against the anthracnose fungus of Asian minor bluegrass (a type of nonhost resistance) is controlled by major gene(s) similar to those involved in the gene-for-gene interactions.

PS12-432

Identification and functional analysis of novel rice blast field resistance gene, *OsXK2b*

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Breeding of crops to harbor field resistance genes, which hardly occurred break-down of resistance, is a pivotal strategy of crop protection. Here, we identified a gene responsible for field resistance to blast disease from rice cv. Habataki, which cause a partial inhibition of hyphal growth of rice blast fungus in rice cells. A high-density linkage map around the locus was constructed and a candidate gene, *OsXK2b*, encoding xylulose kinase was isolated. The candidate gene belonged to the FGGY carbohydrate kinase family as well as *Nho1*, known to need for nonhost resistance of Arabidopsis thaliana. According to DNA sequence variation of *OsXK2b* among various rice cultivars, two missense SNPs were found in the coding region and three haplotypes (Habataki type, Koshihikari type, and Sasanishiki type) containing different pairs of SNPs were present. When degree of rice blast resistance were examined in transgenic rice plants which were overexpressed each haplotype, an overexpressor of Habataki type *OsXK2b* showed strong inhibition of hyphal growth of blast fungus, compared with Nipponbare which are harboring Sasanishiki type. These results strongly suggest that *OsXK2b* is a causal gene of the trait of field resistance. On the other hand, results of xylulose kinase assay of OsXK2b recombinant protein from each haplotype gene revealed that degrees of the resistance and activities of xylulose kinase were negatively correlated. Moreover, *OsXK2b* knockout mutant showed markedly enhanced resistance to the fungus. It is suggested that *OsXK2b* may negatively regulate mechanisms of field resistance against blast fungus in rice plants.

PS12-433

Evolution of the resistance against TMV in *Nicotiana* spp.

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How resistance genes were generated and maintained in wild plant species largely remain unknown. We studied the evolution of resistance against *Tobacco mosaic virus* (TMV) in the *Nicotiana* species. The only known resistance gene against TMV in *Nicotiana* is the *N* gene from *N. glutinosa*. Our mapping results showed that the resistance against TMV in at least other three *Nicotiana* species were also from the *N* locus. However, the all *N* homologues from the three species have less than 95% nucleotide identities with the *N* gene. But the alternative exon is not present in any other *N* homologues. We hypothesize that the resistance in wild *Nicotiana* species were generated independently though they are from the same locus. Two different *N* sequences were found from the seven genotypes of *N. glutinosa*, which exhibit 45 polymorophic sites in the 6,658 bp region. To better understand the mutation of the *N* and *YP* gene (another resistance gene against TMV), the avirulence gene from TMV was transformed into a susceptible tobacco genotype, and the transformant with homozygous avirulence gene was crossed with the TMV resistance genotypes. Approximately 2 million hybrid seeds were screened for each of the two genotypes. Consequently, more than 100 loss of function *N* mutants and 258 loss of function *YP* mutants were discovered. Most of the loss of function of the *N* gene was due to a large deletions spanning the entire *N* gene. The variation of mutation rate between the *N* and the *YP* genes will be discussed.

PS12-434

The chloroplast RECA1 is required for the immune response of Arabidopsis to bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000

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Chloroplast, the descendant of a cyanobacterial ancestor that established symbiotic relationship with ancient eukaryotic host, is involved in various biological processes including biotic stress responses in higher plants. It was investigated whether RECA1, an Arabidopsis chloroplast homolog of bacterial recombinase RecA, might be involved in biotic stress responses in higher plants. First, the RECA1 transcripts were found to be induced in Arabidopsis plants upon treatments with BTH, SA, MeJA, and ethephon, respectively. Microarray experiments showed that RECA1 overexpression changed the expression of numerous genes in Arabidopsis, including the defense-responsive genes that accounted

for about 20% of the up-regulated genes. These defense-responsive genes included a broad range of genes required for plant defense signaling against bacterial pathogen, including the upstream disease resistance genes that encode PAMP- or effector-recognizing factors as well as the resistance-associated signaling or the downstream PR genes. RECA1-overexpressing transgenic plants showed higher levels of SA accumulation than wild-type Arabidopsis, whilst recA1 mutant plants showed opposite results. Consistently, the genes involved in SA biosynthesis were differentially expressed by RECA1. All of these results were in harmony with the resistance of RECA1-overexpressing plants or the susceptibility of recA1 mutant to Pseudomonas syringae pv. tomato DC3000. Genetic experiments further showed that RECA1 plays a role in the NPR1-dependant activation of PR gene expressions. Combined together, it was concluded that chloroplast RECA1 is required for the immune response of higher plants to bacterial pathogen, which is thought to have been developed via inter-organellar signaling involving chloroplast over the course of plant evolution.

PS12-435

Purple acid phosphatase 5 is required for regulation of defense responses against *Pseudomonas syringae* pv. *tomato* in Arabidopsis

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To identify components of plant defense responses, we screened a population of T-DNA mutants in Colombia-0 background for enhanced disease susceptibility to virulent *Pseudomonas syringae pv. tomato* DC3000 (Pst DC3000). We demonstrate that the Arabidopsis Purple Acid Phosphatse 5 (PAP5), induced under prolonged phosphate (Pi) starvation is also required for maintaining basal resistance to certain pathogens. pap5 mutant plants displayed enhanced susceptibility to both virulent and avirulent isolates of bacterial pathogen Pst DC3000 and expression of pathogen inducible gene PR1 was several fold lower than in wild type plants. Similarly, other defense related genes including *ICS1* and *PDF1.2* were also suppressed in pap5 plants. Moreover, treatment of pap5 with BTH (analog of SA) reversed PR1 gene expression. Taken together, these results provide evidences that PAP5 act upstream of SA accumulation to regulate expression of other defense responsive genes in plants infected with Pst DC3000.

PS12-436

The complete genome sequence of Southern rice black-streaked dwarf virus isolated from Vietnam

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We determined the complete genome sequence of a Vietnam isolate of Southern rice black-streaked dwarf virus (SRBSDV). Whole genome comparisons and phylogenetic analysis showed that the genome of Vietnam isolate shared high nucleotide sequence identities of over 97.5% with those of the reported Chinese isolates, confirming a common origin of them. Moreover, the most divergence between different SRBSDV isolates lied in the segments S1, S3, S4 and S6, which was different from the sequence alignment results between SRBSDV and *Rice black streaked dwarf virus* (RBSDV), implying that SRBSDV evolved in a unique way independent of RBSDV. This is the first report of complete nucleotide sequence of SRBSDV in Vietnam and our data provide new clues for further understanding of molecular variation and epidemiology of SRBSDV in Southeast Asia.

PS13-437

Cloning and characterization of a novel canonical rice resistance gene to *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal agent of bacterial leaf blight of rice. The first cloned rice resistance gene to *Xoo*, *Xa21*, encodes a pattern recognition receptor (PRR) RLK that recognizes the PAMP molecule AX21, which is compatible to the Korean strain DY89031 (J18) that contains mutation in AX21 production, resulting in resistance breakdown in the *Xa21*-containging varieties. We propose that durable resistance might be ensured by pyramiding *Xa21* and other typical R genes that recognize type 3 effectors of *Xoo*. With this scenario, we found that a Chinese native variety SKZ exhibits race-specific resistance to DY89031. We mapped the new dominant resistance gene *Xa38(t)* to a 50-kb region on chromosome 3 where no any resistance-related gene has been identified. We found that the insertion of a transposible element results in the functional resistance gene from the non-functional locus. Our current study suggests that DY89031 should secrete an unrecognized Avr effector that is specifically recognized by XA38(t) in SKZ. We also found that XA38(t)-mediated resistance could partially inhibit XA21-miediated resistance probably due to the ETI-PTI interaction.

PS13-438

The GDSL/SGNH lipases OsGL1 and OsGL2 negatively regulate basal immunity in rice

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Lipids and lipid metabolites play important roles in plant-microbe interactions, and lipases catalyze lipid metabolism. However, functions of lipases in plant defense signaling remain largely unknown. Here, we report the identification and functional analysis of two rice lipase genes, *OsGL1* and *OsGL2*, which encode putative GDSL/SGNH lipases. Expression of *OsGL1* and *OsGL2* was suppressed in response to pathogen as well as BTH treatment. *OsGL1* was mainly expressed in leaf and leaf sheath, whereas *OsGL2* showed high expression in the elongating internode and node. Biochemical analysis demonstrated that both OsGL1 and OsGL2 recombinant proteins display lipase activity to hydrolyze p-nitrophenyl acetate and p-nitrophenyl butyrate in vitro. In stable transgenic rice plants, we found that OsGL1 localized to punctate dots resembling lipid bodies while OsGL2 was targeted to the cell wall. To explore the biological functions of *OsGL1* and *OsGL2*, we simultaneously suppressed the expression of both genes and found that *OsGL1/2* RNAi plants displayed enhanced resistance to the bacterial pathogen *Xanthomonas oryzae pv. oryzae*. By contrast, *OsGL1* and *OsGL2* overexpressed plants were more susceptible to the pathogen. Taken together, our results indicate that OsGL1 and OsGL2 are negative regulators of rice basal disease resistance and provide insights into the functions of lipases in plant immunity response.

PS13-439

A rice chitinase-like xylanase inhibitor protein, OsXI1, is related with antifungal activity and plant development

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Previously, we characterized a TAXI type rice xylanase inhibitor gene, *OsXI*1, which was induced by rice blast fungus *Magnaporthe oryzae*. Immunohistochemcal analysis with OsXI1 antibody showed that OsXI1 is highly accumulated in root tissue, especially in the elongation region. However, the purified recombinant protein do not inhibit both β-1,3-xylanase and β-1,4-xylanase activity, indicating OsXI1 does not contain xylanase inhibiting activity. We then confirmed the on gel chitinase activity of recombinant OsXI1, and treatment of OsXI1 caused cell wall degradation of *Rhizoctonia solani*. These data indicated that OsXI1 was related with pathogen defense by its chitinase activity. Furthermore, T-DNA knockout mutant of OsXI1 showed a dwarf phenotype, a different seed shape and root development. We further applied 2-DGE analysis of root tissues of wild type and *OsXI*1 mutant. 2-D close view and 3-D view of each significant regulated protein spots were generated and these spots were identified by MALDI-TOF MS. A calreticulin protein and Calcium-dependent protein kinase gene (*CDPK1*) were not detected in the *OsXI*1 mutant, and stress related proteins phosphoglycerate kinase and chaperonin were increased, indicating that the loss of *OsXI*1 may interfere the intracellular calcium concentration. Additional calcium in the culture medium showed a rescue effect on root length and lateral root development. Taken together, the OsXI1 may a multi-functional protein related with both pathogen defense and plant development.

PS13-440

Connecting pathogen perception to transcriptional reprogramming in plant immune responses

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In plants, defense activation to invading pathogens is mediated by germ-line encoded receptor proteins. Intracellular Nucleotide Binding-Leucine-Rich-Repeat (NB-LRR) receptors can recognize specific pathogen effectors and trigger plant resistance. We aim to learn more about processes connecting immune receptor activation to defense outputs. An important regulatory hub for initiation of defense responses is controlled by the protein EDS1 (Enhanced Disease Susceptibility1). Together with its interaction and signaling partners, PAD4 and SAG101, EDS1 is required for basal defense to virulent biotrophic pathogens and for TIR-NB-LRR (with a Toll-Interleukin1 Receptor domain) triggered resistance. In the TIR-NB-LRR-conditioned immune response, EDS1 operates downstream of receptor activation but upstream of cell death initiation, accumulation of reactive oxygen species, induction of the stress hormone salicylic acid (SA) and transcriptional reprogramming.We have shown that EDS1 shuttles between the cytoplasm and nucleus and that different EDS1 complexes in these compartments cooperate in mediating a complete immune response. To gain further insight into how EDS1 and its signaling partners coordinate multiple defense outputs, transgenic *Arabidopsis* lines were generated in which EDS1 is forced into the nucleus by fusion to a nuclear localization signal (NLS). Plants lacking cytoplasmic EDS1 but expressing high levels of nuclear EDS1 induce defense responses such as SA accumulation and transcriptional reprogramming in the absence of a pathogen trigger. These characteristics of EDS1-NLS lines are dependent on PAD4. Conditional accumulation of nuclear EDS1 via an estradiol-inducible promoter reveals striking differences between immediate and long term effects of nuclear EDS1 which we are now exploring.

PS13-441

Leaf oil bodies produce an anti-fungal compound actively in dying tissues

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Oil bodies are known to function as lipid-storage organelle, which is a passive function. They are present in various cells of many land plants. However, the active functions of oil bodies are not clear, especially in leaves. Here we show that two oil-body-localized proteins, which are induced by senescence and fungal infection, produce an anti-fungal compound. We reveal that oil bodies contained an α-dioxygenase, which is a novel oil-body-localized protein, and a caleosin by proteomic analysis of oil bodies prepared from *Arabidopsis* leaves. Interestingly, after infection with the pathogenic fungus *Colletotrichum higginsianum*, both the α-dioxygenase and the caleosin are induced, and they are targeted to the surface of leaf oil bodies in plant tissues surrounding pathogen infection sites. Recombinant α-dioxygenase and caleosin made an oxygenated fatty acid (oxylipin) from α-linolenic acid (a major lipid component of oil bodies) via an unstable intermediate by a coupling reaction. Importantly, we found that the oxylipin had anti-fungal activity against *C. higginsianum* and *C. orbiculare*. These findings indicate that oil bodies containing the two enzymes function as subcellular factories that produce the anti-fungal oxylipin in response to fungal infection. Interestingly, both the α-dioxygenase and the caleosin are also induced by leaf senescence. Metabolic analysis revealed that senesced leaves contained the oxylipin. We offer oil body-mediated defense which plants might have evolved to prevent fungi from achieving second infection to new healthy plants.

PS13-442

Plant programmed cell death caused by an autoactive form of Prf is suppressed by co-expression of the Prf LRR domain

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In tomato, the NBARC-LRR resistance (R) protein Prf acts in concert with the Pto or Fen kinase to determine immunity against *Pseudomonas syringae* pv *tomato* (*Pst*). Prf-mediated defense signaling is initiated by the recognition of two sequence-unrelated *Pst*secreted effector proteins, AvrPto and AvrPtoB, by tomato Pto or Fen. Prf detects these interactions and activates signaling leading to host defense responses including localized programmed cell death (PCD) that is associated with the arrest of *Pst* growth. We found that Prf variants with single amino acid substitutions at D1416 in the IDH motif in the NBARC domain cause effector-independent PCD when transiently expressed in leaves of *Nicotiana benthamiana*, suggesting D1416 plays an important role in activation of Prf. The N-terminal region of Prf (NPrf) and the LRR domain are required for this autoactive Prf cell death signaling but dispensable for accumulation of the Prf^{D1416V} protein. Significantly, co-expression of the Prf LRR but not NPrf, with Prf^{D1416V}, AvrPto/Pto, AvrPtoB/Pto, an autoactive form of Pto (Pto^{Y207D}), or Fen completely suppresses PCD. However, the Prf LRR does not interfere with PCD caused by Rpi-blb1^{D475V}, a distinct R protein-mediated PCD signaling event, or that caused by overexpression of MAPKKKα, a protein acting downstream of Prf. Furthermore, we found the Prf^{D1416V} protein is unable to accumulate in plant cells when co-expressed with the Prf LRR domain, likely explaining the cell death suppression, despite the underlying mechanism is unknown.

PS13-443

Carbon/nitrogen regulatory ubiquitin ligase ATL31 and ATL6 control the defense response in Arabidopsis

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In higher plants, the metabolism of carbon (C) and nitrogen nutrients (N) is mutually regulated and referred to as the C and N balance (C/N). Plants are thus able to sense and regulate their cellular C/N status to optimize their growth. Arabidopsis *ATL31* and *ATL6* encode a RING-type ubiquitin ligases which play a critical role in the C/N status response (Sato et al. Plant J. 2009). Since many ATL members are involved in the plant defense response, we evaluated whether the ATL31 and ATL6 are involved in defense responses. Our results confirmed that *ATL31* and *ATL6* expression is up-regulated with flg22 as well as with infections with *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*. DC3000). Moreover, transgenic plants overexpressing *ATL31* and *ATL6* displayed increased resistance to *Pst*. DC3000 whereas *atl31 atl6* double knockout mutant resulted in reduced. Further study demonstrated that the expression of *ATL31/ATL6* and defense marker genes was regulated by C/N conditions. Taken together, these results indicate that ATL31 and ATL6 function as key components of both C/N regulation and the defense response in Arabidopsis (Maekawa et al. Plant Mol. Biol. 2012). Relationships between C/N regulation and defense response will be discussed.

PS13-444

Cloning of rice blast resistance gene *Pi34* and comparative analysis to explore a cue of durable resistance

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Rice blast resistant cultivars have been intensively bred by introducing blast resistance genes to rice all over the world. But many complete resistances have been collapsed by new rice blast races within a few years after rice cultivars were spread. In the other hand, partial resistance to rice blast is thought to provide a durable resistance to plants. To understand the mechanism of durable resistance, we cloned the partial resistance gene *Pi34* that was identified from rice cultivar Chubu 32 using QTL analysis (Zenbayashi et al, 2002, 2007), which located in 224 kb genomic region of chr.11. The nucleotide sequence of *Pi34* had no homology with known blast resistance genes and the predicted function of this gene was unknown. We investigated the cytological responses of rice leaf blade cells to rice blast infection using near isogenic lines (NILs), which were introduced chromosomal region harboring *Pi34* or complete resistance gene *Pib*. This comparison of NILs revealed that H_2O_2 accumulation patterns of them were similar in 24 hours post inoculation (hpi), but different in 48 hpi. This finding indicated that presence of H_2O_2 in 24 hpi was not serious factor for partial resistance, but quantitative effect of H_2O_2 thereafter should be clarified by farther analysis.

PS13-445

Qa-SNAREs localized to the trans-Golgi network regulate multiple transport pathways and extracellular disease resistance in plants

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In all eukaryotic cells, a membrane trafficking system connects the post-Golgi organelles, such as the trans-Golgi network, endosomes, vacuoles, and the plasma membrane. This complex network plays critical roles in several higher-order functions in multicellular organisms. The TGN, one of the important organelles for protein transport in the post-Golgi network, functions as a sorting station, where cargo proteins are directed to the appropriate post-Golgi compartments. Unlike its roles in animal and yeast cells, the TGN has also been reported to function like early endosomal compartments in plant cells. However, the physiological roles of the TGN functions in plants are not understood. Here, we report a study of the SYP4 group, which represents the plant orthologs of the Tlg2/syntaxin16 Qa-SNARE that localizes on the TGN in yeast and animal cells. The SYP4 group regulates the secretory and vacuolar transport pathways in the post-Golgi network and maintains the morphology of the Golgi apparatus and TGN. Consistent with a secretory role, SYP4 proteins are required for extracellular resistance responses to a fungal pathogen. We also reveal a plant cell-specific higher order role of the SYP4 group in the protection of chloroplasts from salicylic acid-dependent biotic stress.

PS13-446

A pseudokinase under balancing selection confers quantitative and broad spectrum disease resistance in Arabidopsis

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Pathogens are a threat for crops and natural populations. A major challenge in plant breeding and evolutionary biology is to identify the genetic and molecular bases for natural resistance variation in plant species. The identification of genes underlying natural resistance variation might have enormous practical implications by increasing crop yield and quality, and give fundamental insights in the prediction of evolutionary trajectories of natural populations. We aimed at identifying key genes underlying quantitative resistance in Arabidopsis thaliana to a pathogen species of the bacterial foliar community, i.e. *Xanthomonas campestris* pv. *campestris* (*Xcc*). Black rot of crucifers caused by *Xcc* is possibly the most important disease of crucifers worldwide, and the genetic bases for resistance to this disease are not yet understood. We report the identification, map-based cloning and functional validation of a QTL (QRX3/RKS1) which confers resistance to several *Xcc* races in *Arabidopsis thaliana*. This gene encodes an apparent pseudokinase whose transcription level variation is involved in natural variation of resistance to *Xcc*. The genomic region associated with QRX3/RKS1 was also identified by performing Genome Wide Association (GWA) mapping at different spatial scales, making unequivocally QRX3/RKS1 a major contributor at the species level. Data will be presented concerning the identification, functional analysis and molecular evolution of this novel quantitative resistance gene.

PS13-447

Characterization of constitutively active OsRac1 (CA-gOsRac1) transgenic rice plants generated by gene targeting

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OsRac1 is a member of plant small GTPase Rac/Rop family, which takes two forms in the cells: GDP-bound inactive and GTP-bound

active forms. Previous studies showed that OsRac1 plays a key role in rice immunity by regulating both PAMPs-triggered and effector-triggered immune responses. The constitutively active (CA) G19V mutation of OsRac1 was shown to induce ROS production, phytoalexin synthesis, and defense gene activation leading to resistance to rice blast infection. To further study the effect of the G19V mutation on disease resistance we applied a gene targeting method to generate rice plants whose original OsRac1 locus was modified to the CA form. The targeted CA-OsRac1 gene is termed CA-gOsRac1.We found that transgenic plants carried both wild type and one mutant allele (CA-gOsRac1) in the first generation. This mutation was stably transmitted to the next generation and the mutated gene was expressed at the mRNA level. Levels of mutant transcripts were very low in leaf blade, root and suspension cells but those in leaf sheath and panicle were higher. However, upon chitin treatment, defense-related genes such as *PAL1* and *PBZ1* were more activated in transgenic CA-gOsRac1 compared to wild type. In addition, the induction of cell death was observed in leaf sheath of CA-gOsRac1 plant infected by blast fungus. RNA profiling of *CA-gOsRac1* indicated that it induced genes activated by *M. oryzae* and *X. oo* infection through many signaling processes. These results suggest that CA-gOsRac1 plants showed constitutive immune responses in the absence of infection.

PS13-448

Geraniol synthase whose mRNA is induced by host-selective ACT-toxin in the ACT-toxin-insensitive rough lemon (*Citrus jambhiri*)

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Seven strains of *Alternaria alternata* produce host-selective toxins (HSTs) that are selectively toxic to certain cultivars of plants. HSTs of *A. alternata* are low molecular weight, secondary metabolites with toxicity toward distinct plant genotypes, and have the same specificity as infection by the toxin-producing pathogens. Pathogenicity of *A. alternata* producing HSTs depends on the HSTs. The tangerine pathotype of *A. alternata* causes Alternaria brown spot disease, which affects many tangerine and mandarin cultivars and their hybrids, and the pathogenicity is dependent on the production of ACT-toxin. However, the role of ACT-toxin in ACT-toxin-insensitive plants is currently unknown. Here, we studied the role of ACT-toxin using an ACT-toxin producing *A. alternata* strain SH20 and the ACT-toxin-insensitive plant rough lemon (*Citrus jambhiri*). Induction of some defense related genes in response to SH20 were faster or stronger than in response to the ACT-toxin deficient SH20 mutant. By sequencing subtractive PCR clones obtained from mRNA of rough lemon leaves inoculated with SH20 after subtraction with that of the ACT-toxin deficient SH20 mutant, we isolated the SH20-responsive genes in rough lemon. Among the SH20-responsive genes analyzed in this study, we isolated a terpene synthase gene, *RlemTPS3*. We also determined that RlemTPS3 localizes to the chloroplast and produces the monoterpene geraniol which is released from rough lemon leaves. Geraniol has antifungal activity against *A. alternata*. Therefore, it is suggested that geraniol produced by RlemTPS3 plays important role in rough lemon resistance.

PS13-449

A novel transcription factor, ANAC042, involved in the regulation of camalexin biosynthesis in *Arabidopsis*

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Camalexin is the major phytoalexin in *Arabidopsis*. The camalexin biosynthetic genes have been almost completely elucidated, while molecular mechanisms underlying camalexin induction are still incompletely understood. Here, we report the first characterization study on the involvement of *ANAC042*, a member of NAC transcription factor family genes, in camalexin biosynthesis induction. T-DNA insertion events within *ANAC042* resulted in greatly reduced levels of camalexin production, and enhanced susceptibility to the infection of *Alternaria brassicicola*. Transcript levels of camalexin biosynthetic genes (*CYP71A12*, *CYP71A13*, and *CYP71B15/PAD3*) were greatly lower in the mutants under camalexin induction conditions, indicating that the camalexin defects could be ascribed, at least in part, to the reduced expression levels of these P450 genes. GUS-reporter assays demonstrated differential induction responses of *ANAC042* towards bacterial and fungal pathogens. Particularly, *ANAC042* expression was induced by bacterial flagellin (Flg22) in the root elongation zone, the camalexin biosynthetic site, and the induction was inhibited by adding either a general kinase inhibitor K252a, a Ca2+-chelator BAPTA, or methyl jasmonate. The Flg22-dependent *ANAC042* induction was abolished in ethylene-insensitive *ein2-1* mutant plants, whereas *sid2-2* plants defective for salicylic acid biosynthesis exhibited normal responses, indicating the possible involvement of ethylene signaling in the induction of *ANAC042*. We discuss *ANAC042* as a key transcription factor involved in previously unknown regulatory mechanisms, differentially involved in response to bacterial and fungal pathogen infection, to induce phytoalexin biosynthesis in Arabidopsis.

PS13-450

The anticipation of danger: MAMP perception enhances AtPep-triggered oxidative burst

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The endogenous *Arabidopsis* peptides *At*Peps elicit an innate immune response reminiscent of PTI (pattern-triggered immunity). Detection of various danger signals including microbe-associated molecular patterns (MAMPs) like flg22 leads to elevated transcription of PROPEPs, the *At*Pep precursors, and their receptors. It has been hypothesized that *At*Peps are involved in enhancing MAMP-triggered immunity. Following this idea we analyzed the relationship between MAMP- and *At*Pep-elicited signaling. We found that the perception of MAMPs enhanced a subsequent *At*Pep-triggered production of reactive oxygen species (ROS). Intriguingly, other components of *At*Pep-triggered immunity like Ca^{2+}-influx, MAP kinase phosphorylation, ethylene production and expression of early defense genes and ROS-activated genes remained unchanged. Similarly, we positively correlated the intensities of *At*Pep-triggered response with the abundance of the two *At*Pep-receptors by generating constitutively expressing PEPR1 and PEPR2 transgenic lines and by analyzing *pepr1* and *pepr2* knock out plants. Further we show that enhanced as well as basal ROS production triggered by *At*Peps is absent in the rbohD rbohF double mutant. We present evidence that the enhancement of AtPep-triggered ROS is not based on simple changes in the ROS detoxification machinery and is independent of MAP kinase and Ca^{2+} signaling pathways. Taken together we suggest how potential functions for the enhancement of *At*Pep-elicited ROS by previous MAMP perception: First, the strong ROS release might impair microbial growth in areas of *At*Pep release, and second, ROS triggered by *At*Peps might take part in ROS mediated systemic signaling in the case of danger.

PS13-451

Imaging analysis of mitochondrial movement in rice cells during rice *Magnaporthe oryzae* interactions

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We produced transgenic rice plants in which some organelles, including mitochondria, were labeled with green fluorescent protein to analyze organelles changes during the early infection process. Microscopic observations of rice sheathes inoculated with *Magnaporthe oryzae* revealed that mitochondrial movement alters around the infection sites in incompatible interactions. Two types of alteration of mitochondrial distribution were observed. In the cells contacting with an appressorium, mitochondria radially translocated toward the appressorium (type-I distribution). In the cells around the invaded cell, mitochondria translocated to the side close to the invaded cell (type-II distribution). The remarkable type-II distribution pattern was observed after the adjacent cell showed hypersensitive reaction. Neither type of mitochondrial distribution pattern was observed when inoculated with *M. oryzae* mutants deficient in penetration: the *sdh* mutant, in which appressoria cannot mature, and the *mst12* mutant, which cannot develop penetration pegs. This result indicates that alteration of mitochondrial distribution in a rice cell requires the fungal penetration into the nearby cells. The alteration of mitochondrial distribution was not observed in compatible interactions, however, the cells infected by the *ssd1* mutant, which elicits the host defense response involving hypersensitive reaction-like browning of the penetrated cells, showed both types of mitochondrial distribution pattern in both compatible and incompatible interactions. These results suggest that the alteration of mitochondrial distribution in rice cells after inoculation with *M. oryzae* is closely related to the hypersensitive reaction in rice cells.

PS13-452

PAMP-mediated pathogen defense in *Solanum tuberosum*

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The hemibiotrophic oomycete *Phytophthora infestans* is responsible for late blight of potato (*Solanum tuberosum*). In susceptible potato plants, the pathogen-associated molecular pattern (PAMP) Pep-13 from *Phytophthora* induces enhanced resistance. Pep-13 is a 13 aa large peptide motif located near the C-terminus of a cell wall transglutaminase from *Phytophthora* species. The application of Pep-13 induces the accumulation of jasmonic acid (JA), salicylic acid (SA) and hydrogen peroxide, as well as the activation of defense genes and hypersensitive cell death. Both JA and SA have been shown to be required for successful activation of the Pep-13 mediated defense responses. To investigate the molecular mechanisms of PAMP-induced defense responses in susceptible potato plants, a candidate gene approach was performed. Using microarray analyses, we identified more than 700 Pep-13 activated genes, 50 of which are JA-dependently expressed. Functional analyses are performed using RNA interference constructs to down-regulate the expression of specific candidate genes. Possible changes in the pathogen response of these transgenic plants will be assessed by analyzing the response to Pep-13 and to infection by *P. infestans*.

PS13-453

Climate change effects on the interaction between barley and two fungal pathogens with opposite lifestyles

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The predicted changes in the world climate are believed to affect the physiology of plants, and their interaction with pathogens. It is generally hypothesized that crop plants may become more prone to diseases in the future, but it is difficult to generalize, and few studies have been conducted, where more than two climatic factors are changed simultaneously. Furthermore the mechanisms behind the observed and predicted changes in susceptibility are not understood. Here we examine, how factors associated with climate change are affecting disease severity and resistance in barley (*Hordeum vulgare*) towards two fungal pathogens with opposite lifestyles: the biotrophic *Blumeria graminis* f.sp. *hordei* (powdery mildew), and the hemibiotrophic *Bipolaris sorokiniana* (spot blotch). Plants are grown in a phytotron with different levels of temperature, [CO_2] and [O_3] either as single factors or in combination, resembling the conditions in 2075 as expected by IPCC. Leaves were assessed for the diseases either visually, microscopically and/or by qPCR. We found that disease development of powdery mildew and spot blotch was effected in opposite ways in the different climatic conditions. Elevated [O_3] and temperature increased penetration resistance towards powdery mildew, while symptoms development of spot blotch were promoted. However, when plants were exposed to elevated temperature, [CO_2] and [O_3] simultaneously, infection increased to levels higher than ambient for both pathogens.In order to understand the molecular and biochemical changes responsible for our observations, we are currently making whole transcriptome and untargeted metabolomic analyses of barley grown in the climatic conditions with and without infection.

PS13-454

***Medicago truncatula* as a model to study vascular wilt disease: genetic traits and regulatory mechanisms**

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Vascular wilt disease caused by bacteria or fungi causes crop losses worldwide. We found that *Ralstonia solanacearum* (*Rs*)[Vailleau et al.,2007] and *Verticillium albo-atrum* (*Vaa*) [unpublished] infect the model legume plant *Medicago truncatula*. These pathosystems can be used to study cross-talk between pathogenic and symbiotic interactions. Moreover, line A17 is respectively susceptible to *Rs* and resistant to *Vaa*, whereas line F83005.5 shows opposite responses. Thus the two pathosystems can be used to study genotype-dependent regulation of defence responses against vascular pathogens.A core collection of *M. truncatula* lines showed wide diversity of the response to *Vaa*, from highly susceptible to fully resistant. Major QTLs involved in tolerance to *Vaa* were identified on chromosomes 2 and 7 respectively in 2 different crosses. These QTLs do not co-localise with identified *Ve* gene homologs. Resistant A17 plants eliminate the fungus from their vessels 5 to 7 days after inoculation. Inoculation of nodulation mutants with *Vaa* and *Rs* indicates that regulatory mechanisms of symbiosis might also be involved in pathogenic interactions. The role of phytohormones was studied by external treatments before inoculation.To study regulatory RNAs involved in the response to vascular wilt pathogens libraries of small RNAs were produced from roots of lines A17 and F83005.5, inoculated with *Rs* or *Vaa* (MirMed project). Comparative analysis of the libraries allows to identify miRs associated to a pathosystem and to resistance. A pilot experiment with miR393 suggests that this microRNA which controls auxin signaling may be involved in the regulation of root responses to vascular wilt.

PS13-455

Cross-talk between *AtNHR2A* and *AtNHR2B* to modulate nonhost defense responses in *Arabidopsis*

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NHR2 was found in *N. benthamiana* through a virus-induced gene silencing (VIGS)-mediated fast-forward genetics screen aimed at identifying genes involved in nonhost disease resistance. Silencing of *NHR2* in *N. benthamiana* allowed growth of nonhost pathogens *Pseudomonas syringae* pv tomato T1 and *P. syringae* pv glycinea and enhanced susceptibility towards the host pathogen, *P. syringae* pv. tabaci. *NHR2* does not have homology to any genes with known function in the NCBI database. We identified two homologs in Arabidopsis that we named *AtNHR2A* and *AtNHR2B*. T-DNA mutants of both *AtNHR2A* and *AtNHR2B* compromised nonhost resistance against nonhost bacterial pathogens, *P. syringae* pv. tabaci and *P. syringae* pv. phaseolicola, and showed increased susceptibility to a host pathogen, *P. syringae* pv. maculicola. We found that in spite of a 60% identity at amino acid level, *AtNHR2A* and *AtNHR2B* are not redundant as these genes have different patterns and levels of gene expression upon inoculation with the nonhost and host pathogens. Comparison of expression levels of several defense-related genes in wild-type Col-0, *Atnhr2a* and *Atnhr2b* mutants after inoculation with nonhost and host pathogens revealed different patterns of gene expression. Furthermore, by examining the expression of *AtNHR2A* and *AtNHR2B* in publicly available microarray data we found high levels of expression upon certain hormone treatments. We propose that *AtNHR2A* and *AtNHR2B* play significant roles in transducing or propagating hormonal signals to achieve defense responses during plant-microbe interactions.

PS13-456

ROS, a two-faced Janus in plant responses to pathogens?

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Reactive oxygen species (ROS) are produced at and around infection sites during plant defense responses. NADPH oxidases, also referred as respiratory burst oxidases homologues (RBOH) have been shown to play an important role in ROS production in plants. Among 10 RBOH genes (A-J) in *Arabidopsis thaliana*, AtRBOHD and AtRBOHF are known to be involved in defense responses. The cyst nematode *Heterodera schachtii* infects roots of *Arabidopsis* plants and parasitizes by modifying root cells to a hypertrophic syncytial feeding cell system.The aim of this work is to understand the role of AtRBOH-mediated ROS during plant-nematode interaction. Visualization of ROS production using DAB (Diaminobenzidine), CM-H2DCFDA and transgenic plants encoding H2O2 sensor HyPer revealed a distinct pattern during migration, syncytium induction, and feeding. Our results suggest that AtRBOHD and AtRBOHF are required for this pathogen-induced ROS production. Unexpectedly, knock-out mutation of AtRBOHD/F reduced development of female nematodes by 90%, a situation resembling incompatibility. Treatment of plants with DPI (diphenylene iodonium), an inhibitor of NADPH oxidase, gave similar results. Similarly, overexpression of *AtRBOHD* increases the suitability of plants to nematodes. Further analyses of atrbohd/f revealed up-regulation of plant defense response genes (*WRKY33, PR1, PR2, PR3* and *PR5*) in syncytia but no change in the expression of anti-oxidant genes (*APX1, CAT1, GR1*). Taken together, our findings suggest a novel role of AtRBOH-mediated ROS in the function of compatible plant-pathogen interactions. Molecular mechanisms underlying this role will be discussed in detail.

PS13-457

A sec14P phospholipids transfer protein regulates plant immunity in *Nicotiana* plants

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Ralstonia solanacearum causes bacterial wilt in several economically important solanaceous. To elucidate the molecular mechanisms of plant-*R. solanacearum* interactions, we isolate and analyze *R. solanacearum*-responsive genes from *Nicotiana* plants. In this report, we focused on *NbSec14P* with similarity to *sec14p* from yeast. *Nbsec14P* rescued growth of temperature-sensitive *sec14p* mutant of yeast and extracellular secretion of invertase from the mutant yeast. Recombinant Nbsec14P showed phosphatidylinositol and phospahtidylchorine transfer activity. Expression of *Nbsec14P* was strongly induced in tobacco leaves inoculated with avirulent strain of *R. solanacearum* 8107, and was slightly enhanced by the inoculation with virulent strain of *R. solanacearum* OE1-1. In *Nbsec14P*-silenced *N. benthamiana* plants, expression of defense-related genes was compromised, and growth of *R. solanacearum* was significantly accelerated. Moreover, disease development caused by *R. solanacearum*, was accelerated in the silenced plants. Intriguingly, changes of phospholipid contents were observed in *Nbsec14P*-silenced plant. These results suggested that Nbsec14P have a role in the defense responses through the regulation of phospholipid metabolisms in *Nicotiana* plants.

PS13-458

Functional analysis of the elicitor-inducible bZIP transcription factor OsTGAP1 in rice

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Plants attacked by pathogenic microorganisms respond with a variety of defensive reactions, including the production of phytoalexins. We previously showed that biosynthetic genes for momilactones and phytocassanes, major diterpenoid phytoalexins in rice, are respectively clustered on rice chromosome 4 and chromosome 2. We also showed that an elicitor-inducible bZIP transcription factor, OsTGAP1, is involved in the regulation of the expression of almost all the biosynthetic genes for diterpenoid phytoalexins including the methylerythritol phosphate (MEP) pathway genes. Here we performed chromatin immunoprecipitation with next-generation sequencing technology (ChIP-seq) to elucidate the OsTGAP1 binding sites. As a result, approximately 2,700 binding sites were identified under the untreated and elicitor-treated conditions, respectively. We found approximately 1,200 genes whose transcription start sites are located within 2 kb from the OsTGAP1 binding sites under the both conditions. According to our previous transcriptome analysis, the expressions of around one-sixth genes of the above 1200 genes were changed in the OsTGAP1 over-expressing cells compared to wild-type cells, indicating that these genes are promising candidates of the OsTGAP1 target genes. However, OsTGAP1 did not bind to the upstream regions of the majority of diterpenoid phytoalexin biosynthetic genes including the MEP pathway genes, and predominantly bound to the inetergenic regions and both ends of phytoalexin biosynthetic gene clusters. Among the direct target genes, we focus on the *OsDXS3* gene in the MEP pathway and its transcriptional regulatory mechanism by

OsTGAP1 is now under investigation.

PS13-459

Molecular mapping of *Rmo2*, a core locus conditioning the resistance of barley to various host-specific subgroups of *Magnaporthe oryzae*

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Magnaporthe oryzae is the most common fungal species among causal agents of blast diseases of gramineous plants. This species is found on various crops e.g., foxtail millet, rice, oat, and wheat, but isolates from each crop are almost exclusively pathogenic on their original host genus. Barley (*Hordeum vulgare*) is a staple crop belonging to Triticeae. The relationship between barley and *M. oryzae* isolates is very complex. Barley-specific isolates or subgroup have not been recognized so far. To characterize the relationship between barley and *M. oryzae*, 24 barley cultivars were inoculated with 16 isolates from various hosts. These interactions included various types from nonhost-like immune responses through typical host responses. Genetic mechanisms of the resistance of five representative barley cultivars to various subgroups of *M. oryzae* were examined using cv. Nigrate, which was highly susceptible to all the isolates, as a common parent of genetic crosses. The resistance of all five cultivars was attributed to a single, identical locus, which was designated as *Rmo2*. Nevertheless, the *Rmo2* locus in each cultivar showed different range of resistance reaction to isolates (subgroups). This locus was mapped on the chromosome 7H. A fine-map around *Rmo2* was constructed using recombinants that were selected with two flanking EST markers, k10750 and k8512, from 2,497 susceptible (*rmo2rmo2*) plants. The *Rmo2* locus was limited in a region corresponding to 44 recombinants. The region corresponded to the genome sequence of *Brachypodium distachyon*, which spanned 129 kb.

PS13-460

***Arabidopsis* NSL2-related cell death is induced by ROS production from chloroplasts**

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Plants are remarkable organisms since they constantly adjust their growth and developmental patterns in response to changes in their environment. One of the mechanisms that adjust to the environmental changes, cell death is known for resistance to pathogen attack. Arabidopsis mutant *nsl2* (necrotic spotted lesion 2; also reported as the *cad1*) which showed hypersensitive response seen in the lesion mimic mutants. In addition, the *nsl2* mutant showed promotion of *PR* gene transcription and accumulation of SA and JA. Inoculation of the mutant with virulence pathogen also showed restriction of bacterial growth (Plant Cell Physiol. 2005, 46: 902-912). Recently we demonstrated that the *nsl2* mutant accelerates senescence in the dark conditions. In connection with accelerated the senescence, chlorophyll catabolic enzymes were accumulated in the *nsl2* mutant than in the wild type. More over, microscopic analysis of oxidant formation using reactive oxygen species (ROS)-dependent fluorescent probe showed that these reactive species are accumulated in chloroplasts in the mutant leaves. These results suggest that the NSL2-related cell death is induced by ROS produced from the chloroplasts.

PS13-461

Application of D-allose for disease control of rice bakanae disease: Sugar phosphorylation of D-allose by hexokinase gives GA-dependent shoot growth inhibition

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D-Allose has an inhibitory effect on shoot growth in rice. A microarray analysis revealed that D-allose treatment induces defense-related genes, and D-allose confers resistance to bacterial blight pathogen in rice. D-allose was also the most effective inhibitor of plant growth among all hexose sugars tested. To clarify the overall mechanism of the D-allose effects in plants, we examined a possible involvement of the hexokinase <HXK>-dependent pathway and suppression of the gibberellin <GA>-signaling to explain the growth inhibition caused by D-allose. D-Allose strongly inhibited the GA-dependent responses such as elongation of the second leaf sheath and induction of alpha-amylase in embryo-less half seeds in rice. The growth of the slender rice1 <*slr1*> mutant, which exhibits a constitutive GA-responsive phenotype, was also inhibited by D-allose, and the growth inhibition of the *slr1* mutant was also abolished by HXK-inhibitor. The *Arabidopsis glucose-insensitive2* <*gin2*> mutant, which is a loss-of-function mutant of the glucose sensor AtHXK1, showed a D-allose-insensitive phenotype. D-allose treatment to the transgenic *gin2* overexpressing $AtHXK1^{WILD}$ or $AtHXK1^{S177A}$ revealed that phosphorylation of D-allose by HXK is an important process for the D-allose-induced growth inhibition. Furthermore, ABF1 and ABI5 <ABA signaling factor> were also up-regulated HXK-dependently in D-allose-treated rice and *Arabidopsis*. On the basis of these results, we tried the control of bakanae disease, which shows abnormal shoot elongation caused by fungal pathogen-produced GA, and application of D-allose successfully suppressed the disease symptoms.This study was supported by Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

PS13-462

Overexpression of tobacco Dof transcription factor enhances transcriptional activation of the virus resistance gene *N* and ROS generation

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Samsun NN tobacco (*Nicotiana tabacum*) carrying the virus resistance gene *N* shows hypersensitive response (HR) against *Tobacco mosaic virus* (TMV) infection. The helicase domain (p50) of the virus replicase acts as an elicitor and upregulates the *N* transcription prior to HR induction. We have found that an upstream region of *N* contains binding motifs of Dof transcription factors (BBF proteins of tobacco) and that the co-expression of p50 and BBF1 induces HR more effectively than the expression of p50 alone. In this study, we further investigated the functional involvement of BBF1 in *N* transcription and HR induction in Samsun NN. We examined the effects of the overexpression of BBF1/p50 or TMV infection on transcription levels of the endogenous *N* and BBF1 genes. The expression of BBF1 alone enhanced the transcription level of the *N* gene. The co-expression of p50 and BBF1 advanced the timing of transcriptional activation of the N gene. Either the expression of p50 or TMV inoculation had no

influence on the transcription level of the endogenous *BBF1* gene. Reporter assays by using the upstream region of the *N* gene revealed that the overexpression of BBF1 resulted in the transcriptional enhancement even in the Samsun nn tobacco lacking the *N* gene. We also found that reactive oxygen species (ROS) levels increased under the overexpression of BBF1 regardless of the co-expression of p50. Our data suggest that BBF1 may play important roles in N-mediated HR induction through stimulation of *N* transcription and ROS generation.

PS13-463

Phosphorylated D-allose confers disease resistance with ROS generation in Rice

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Rare sugars are defined as monosaccharides with low abundance in nature. D-Allose at 5 mM among several sugars conferred the abilities for induction of disease resistance by a leaf inoculation test using rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). D-Allose-treated rice exhibited a lesion mimic formation on the leaves with an accumulation of reactive oxygen species (ROS). The D-allose-mediated induction of ROS generation, subsequent lesion mimics development and resistance to the rice bacterial blight pathogen were suppressed by treatment with a hexokinase inhibitor of N-acetyl-D-glucosamine. 6-Deoxy-D-allose which is a structural derivative of D-allose at the carbon 6 position of phosphorylation site did not confer resistance to *Xoo*. In addition, a peak of D-allose 6-phosphate (A6P) was detected in the extracts from D-allose-treated rice leaves, but not in those from mock-treated leaves. We characterized the kinase activity to D-allose of two main rice hexokinases, HXK5 and HXK6, using respective recombinant HXK5 and HXK6 by HPLC detection of A6P. Transgenic rice plants constitutively expressing *Escherichia coli AlsK* encoding D-allose kinase to increase D-allose 6-phosphate synthesis also showed enhanced sensitivity to D-allose. These results indicated that D-allose is the first monosaccharide discovered to have an ability for induction of ROS generation, subsequent lesion mimics development, PR-protein gene expression, and resistance to *Xoo* by hexokinase-mediated conversion of D-allose to D-allose 6-phosphate.This study was supported by Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

PS13-464

Isolation and identification of natural diterpenes that inhibit bacterial wilt disease in tobacco, tomato, and *Arabidopsis* and analysis of their mode of action

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The soil-borne bacterial pathogen *Ralstonia solanacearum* invades a broad range of plants through their roots, resulting in wilting of the plant. Two wilt disease-inhibiting compounds were biochemically isolated from tobacco (*Nicotiana tabacum*) and identified as labdane-type diterpenes. When exogenously applied to their roots, these two diterpenes inhibited wilt disease caused by *R. solanacearum* in tobacco, tomato, and *Arabidopsis* plants without exhibiting any antibacterial activity. Microarray analysis identified many diterpene-responsive genes in *Arabidopsis* roots, including genes encoding or with a role in ATP-binding cassette (ABC) transporters, biosynthesis and signaling of defense-related molecules, and signal transduction cascade components. Inhibition of wilt disease by these diterpenes was attenuated in some defense-related *Arabidopsis* mutants. These results suggest that multiple host factors are involved in the inhibition of bacterial wilt disease by diterpenes.

PS13-465

Dispersed benzoxazinone gene cluster: Molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye

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Benzoxazinones (Bxs) are major defensive secondary metabolites in wheat (*Triticum aestivum*), rye (*Secale cereale*) and maize (*Zea mays*). Here, we identified full sets of homoeologous and paralogous genes encoding Bx glucosyltransferase (GT) and Bx-Glc glucosidase (Glu) in hexaploid wheat (2n=6x=42, AABBDD). Four *GT* loci (*TaGTa-TaGTd*) were mapped on chromosomes 7A, 7B (two loci) and 7D, whereas four *glu1* loci (*Taglu1a-Taglu1d*) were on chromosomes 2A, 2B (two loci) and 2D. Transcript levels differed greatly among the four loci; B-genome loci of both *TaGT* and *Taglu1* genes were preferentially transcribed. Catalytic properties of the enzyme encoded by each homoeolog/paralog also differed despite high levels of identity among amino acid sequences. The predominant contribution of the B genome to GT and Glu reactions was revealed, as observed for the five Bx biosynthetic genes, *TaBx1-TaBx5*, which are separately located on homoeologous groups-4 and -5 chromosomes. In rye, where the *ScBx1-ScBx5* genes are dispersed to chromosomes 7R and 5R, *ScGT* and *Scglu* were located separately on chromosomes 4R and 2R, respectively. The dispersal of Bx-pathway loci to four distinct chromosomes in hexaploid wheat and rye suggests that the clustering of Bx-pathway genes, as found in maize, is not essential for coordinated transcription. On the other hand, barley (*Hordeum vulgare*) was found to lack the orthologous *GT* and *glu* loci despite its close phylogenetic relationship with wheat and rye. These results contribute to our understanding of the evolutionary processes that the Bx-pathway loci have undergone in grasses.

PS13-466

Identification of molecules that modulate pathogen induced programmed cell death in *Arabidopsis*

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Effector triggered immunity in plants is often associated with a local programmed cell death in the infected tissue known as hypersensitive response (HR). HR lesions caused by the avirulent bacterium *Pseudomonas syringae* in *Arabidopsis* is typically initialized at a single cell and then spread to surrounding mesophyll cells. Thus, a signal that can propagate the cell death from the infection site to neighboring cells must be released. We sought to find diffusible signaling compounds released by pathogen

challenged plants that could cause cell death in naive tissue. A plant line heterologously expressing the bacterial effector AvrRpm1 under the control of a dexamethasone inducible promoter was used to scale up the HR. Organic low molecular weight compounds released by the plant after effector elicitation were purified and fractionated by reverse phase HPLC. The fraction that had the strongest cell death promoting activity when infiltrated into naive tissue was further analyzed by GC-MS. In this fraction, a single compound could be indentified and structure determined. We can now provide three lines of evidence that the isolated compound is a modulator of cell death in Arabidopsis: (i) the compound is synthesized and released by tissue undergoing effector triggered cell death (ii) the pure substance causes lesions and necrotic spots when infiltrated into naive tissue; (iii) plants impaired in the synthesis of the compound show reduced level of cell death induced by *P. syringae* and resistance against *Hyaloperonospora arabidopsides*.

PS13-467

Ergosterol perception in plant systems: an "omics" approach

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Plants are subject to continuous attack by a variety of microorganisms, including phytopathogenic fungi, within their environment. An interesting MAMP is the fungal sterol, ergosterol, of which the receptor(s) and induced signal transduction pathway(s) have not yet been elucidated, but has been shown to trigger defense responses in tomato and tobacco cells, as well as the expression of PR-14, a lipid transfer protein (LTP), in grape-vine cells and up-regulation of an oxysterol-binding protein (OSBP) in potato. Here, we utilized an "omics" approach to elucidate the effect of ergosterol on both tobacco (*Nicotiana tabacum*) and Arabidopsis (*Arabidopsis thaliana*) plant systems with varying concentrations (0 - 1000 nM) over a time period (0 - 24 h). The results showed differential changes in the metabolome of tobacco cells, leading to variation in the biosynthesis of secondary metabolites, with five bicyclic sesquiterpenoid phytoalexins namely capsidiol, lubimin, rishitin, solavetivone and phytuberin identified as being ergosterol-induced, and contributing to the altered metabolome (Tugizimana *et al*., 2012). Ergosterol is also able to trigger changes to the transcriptome in Arabidopsis as investigated using ACP-DDRT-PCR. LTP (*At4g12470*), OSBP (*At4g08180*) and PR-1 (*At2g14610*) showed transient induction typical of defense genes. Preliminary qPCR experiments indicate that the *18S rRNA* gene of Arabidopsis is not suitable as a reference candidate since its expression is altered through treatment with 250 nM ergosterol at various times, while the SAND family protein and Elongation Factor 1 alpha showed stable expression profiles at different treatment-time intervals. Currently, this research is being complemented proteomically by dendrimer-based phosphoproteomics.

PS13-468

Proteasome transformation in response to pathogen attack

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Proteasome is a large multisubunit complex that degrades damaged or ubiquitinated proteins, archiving protein-quality control and fine-tuning of amount of the specific target proteins. Interestingly, the most subunits are encoded by duplicated genes in higher plant while specific functions of each paralogous subunit have been still unclear. We have demonstrated that loss-of-function mutant of the specific paralog shows aberrant response to multiple stress conditions. Furthermore, our recent data showed that each peptidase activity is affected by flg22 treatment, suggesting that plant proteasome is transformed in the structures and functions in response to environmental stress conditions, as well as the immunoproteasome and thymoproteasome reported in mammals. To evaluate this hypothesis, we tried to identify subunit composition of proteasome with the affinity purification and MS analysis. Possibility of plant proteasome transformation in response to pathogen attack will be discussed.

PS13-469

MAMP-responsive phosphoprotein RAM1 negatively regulates ROS production in Arabidopsis

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Phosphorylation plays critical roles in MAMP (microbe-associated molecular patterns)-triggered immunity. Therefore, we examined phosphoproteome changes in Arabidopsis upon MAMP (microbe-associated molecular pattern) treatment to identify novel players involved in MAMP signal transduction. As a result, we identified 569 proteins whose phosphorylation status significantly changed in response to flg22 and/or chitin treatments. To verify involvement of the identified proteins in MAMP-triggered responses, we have been isolating T-DNA insertion lines for these proteins and characterizing flg22-induced ROS (reactive oxygen species) production in the isolated mutants. So far we have identified 38 genes as regulators of flg22-induced ROS production and named these genes *RAM (ROS abnormal production mutant)*. Among the *RAM* genes, *RAM1* encodes an unknown protein and *ram1* plants show enhanced ROS production in response to flg22 and elf18 treatments. Enhanced ROS production in *ram1* plants were also observed upon an avirulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 *avrRpm1* infection. Interestingly, chlorotic cell death was observed in *ram1* plants after *Pseudomonas syringae* pv. *tomato DC3000 hrcC*$^-$ infection. Gene expression analysis revealed that defense-related genes are up-regulated in *ram1* plants. These results suggest that RAM1 functions as a negative regulator of MAMP-triggered immunity in Arabidopsis.

PS13-470

Plants growth promotion by *Streptomyces*. ll. Involvement of plant pathway

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Two polyphenol oxidases (PPOs) produced by *Streptomyces* exhibit opposite effects on plant growth promotion, *i.e.*, extracellular MelC2 enhances plant growth, whereas intracellular MelD2 diminishes it. To investigate the physiological pathways of plants involved in these effects, we performed microarray analysis of *Arabidopsis thaliana* Col-0 seedlings inoculated with different *melC* and *melD* strains of *Streptomyces*. The results showed that the presence of *melC* and the absence of *melD* in *Streptomyces* similarly induced or repressed certain circadian clock related genes, *TOC1*, *CCA1, COR27, COL9* and *PRR3* in *Arabidopsis*. To confirm their involvement, three circadian clock mutants (*toc1, cca1, and cor27*) of *Arabidopsis* were tested. The growth promotion effects of the PPOs was insignificant in all three mutants. Moreover, growing *Arabidopsis* under a short daytime (8-h light/16-h dark) instead of long daytime (16-h light/8-h dark) also eliminated the growth promotion effects. To test the involvement of the plant growth promotion rhizobacterium-potentiated induced systemic resistance (ISR), two mutants of *Arabidopsis, ein2-1* (ethylene insensitive) and *jar1-1* (methyl jasmonate insensitive), were tested for the growth promotion effects The results showed that the ethylene pathway was involved in the effect of MelD2 (but not MelC2), and the jasmonic acid pathway was involved in the effect of MelC2 (but not MelD2). This indicated that the two PPOs act through ISR in separate pathways.

PS13-471

Plants growth promotion by *Streptomyces*. I. Involvement of polyphenol oxidases of *Streptomyces*

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Filamentous bacteria *Streptomyces* are among the most abundant bacteria in soil. They play an important role as scavengers, degrading organic wastes. While a small fraction of *Streptomyces* is plant pathogens, many establish rhizospheric or endophytic associations with healthy plants often with beneficial effects, presumably resulting from (iii) direct promotion of plant growth, (ii) reduction of infection by pathogens, or (iii) promotion of plant-microbe symbioses. In rhizosphere, *Streptomyces* face a wide range of plant phenolics. These phenolics are substrates for polyphenol oxidases (PPO) produced by two homologous PPOs of Streptomyces - a universally present intracellular MelD2 and a sporadically present extracellular MelC2. While MelD2 appears to play a defensive role and decreases the toxicity of phenolics (presumably by replacing the spontaneous ROS-generating oxidation of phenolics intracellularly), MelC2 increases the sensitivity (presumably by converting the phenolics extracellularly into more permeable hydrophobic quinones). Interestingly, these two PPOs also exhibit opposite effects in promoting plant growth, i.e., MelC2 increases it, and MelD2 reduces it. This opposite effect was observed in eight of eleven plants tested in the soil. The same effects were also observed in *Arabidopsis* growing on agar, ruling out the involvement of other microbes and the processing of soil materials by extracellular enzymes of Streptomyces. The latter was supported by the lack of effect of mutations in the major protein secretion pathway (Tat) of *Streptomyces* on the growth promotion. The investigation of the pathways involved in the observed growth promotion effects is presented in the accompanying poster.

PS13-472

MAPK cascades control *NbRBOHB* promoter activity in *Nicotiana benthamiana*

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Pathogen-induced ROS burst is mainly caused by activation of an NADPH oxidase in plasma membrane. Plant NADPH oxidases are designated as RBOH (respiratory burst oxidase homolog). In *Nicotiana benthamiana* leaves, the ROS burst is caused by NbRBOHB and occurs in two phases, rapid phase I and massive phase II bursts. The plant innate immune system consists of two layers. First layer relies on the perception of pathogen-associated molecular patterns (PAMPs). The responses to PAMPs are called PAMP-triggered immunity (PTI). The second layer is the recognition of pathogen effectors, which can promote pathogen fitness thorough host resistance (R) proteins. Effector recognition by R protein leads to effector-triggered immunity (ETI). ETI is an accelerated and magnified defense response compared to PTI, and is often accompanied by localized cell death termed a hypersensitive response (HR). Here, we investigated *NbRBOHB* gene expression and its promoter activity in PTI (flg22 and INF1), ETI (AVR3a/R3a) and in response to $MEK2^{DD}$ that constitutively activates SIPK and WIPK. It is well known that INF1 induces strong defense response compared to flg22, and is accompanied by HR cell death. *NbRBOHB* gene was induced by INF1, AVR3a/R3a and $MEK2^{DD}$, but not by flg22 at 24 h after the treatments. The same is true for the *NbRBOHB* promoter activity. These results suggest that *NbRBOHB* could be robustly induced by INF1 and Avr3a/R3a via MAPK in association with phase II burst.

PS13-473

Molecular characterization and regulation of a Nicotiana tabacum S-domain receptor-like kinase gene induced during an early rapid response to lipopolysaccharides

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An S-domain receptor-like kinase (RLK) gene in Nicotiana tabacum, responsive to lipopolysaccharide (LPS) elicitation, was identified. The gene, corresponding to a differentially expressed LPS-responsive EST, was fully characterized to investigate its involvement in LPS-induced responses. The full genomic sequence, designated Nt-Sd-RLK, encodes for a S-domain RLK protein containing conserved modules (B-lectin-, S- and PAN-domains) reported to function in mediating protein-protein and protein-carbohydrate interactions in its extracellular domain, as well as the molecular architecture to transduce signals intracellularly through a Ser/Thr kinase domain. Phylogenetic analysis clustered Nt-Sd-RLK with S-domain RLKs induced by bacteria, wounding and salicylic acid. Perception of LPS induced a rapid, bi-phasic response in Nt-Sd-RLK expression with a 17-fold up-regulation at 3 and 9 h. A defense-related W-box cis element was found in the promoter region of Nt-Sd-RLK and the transient induction of Nt-Sd-RLK in cultured cells by LPS exhibited a pattern typical of early response defense genes. Nt-Sd-RLK was also responsive to salicylic acid induction and was expressed in differentiated leaf tissue, where LPS elicited local as well as systemic up-regulation. The results contribute new knowledge about the potential role that S-domain RLKs may play within interactive signal transduction pathways associated with immunity and defense.

PS13-474

The role of a splice variant product of the virus resistance gene *N* in the induction of hypersensitive response

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The tobacco *N* gene, which confers resistance to *Tobacco mosaic virus* (TMV), is transcriptionally induced by the infection of TMV. The N protein recognizes the helicase domain (p50) of TMV replicase as an elicitor and induces hypersensitive response (HR) that results in programmed cell death leading to the restriction of the spread of TMV. The *N* gene consisting of TIR, NBS and LRR domains produces two transcripts, N_S and N_L, by alternative splicing. The N_S and N_L transcripts encode the full-length N protein and the truncated N protein (Ntr) lacking most of the LRR region, respectively. In this study, we analyzed the biological role of Ntr in the N-mediated hypersensitive cell death. In Samsun nn tobacco plants lacking the *N* gene, HR was induced by the expression of both N and p50. However, it was suppressed by the additional expression of Ntr. HR was also suppressed by the expression of both p50 and Ntr in Samsun NN tobacco plants carrying the *N* gene. The endogenous *N* gene expression was not suppressed by expression of Ntr with or without p50-mediated HR induction. Our data indicate that Ntr does not influence the endogenous *N* expression but it causes dominant negative effects on the recognition of the elicitor p50 or signal transduction pathways promoting HR.

PS13-475

Mapping PAMP Responses in *Brassicas*

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Recent work in plant-pathogen interactions has led to the discovery of pattern recognition receptors (PRRs) which confer a rapid early immune response by recognising pathogen-associated molecular patterns (PAMPs). PAMP-triggered immunity (PTI) could contribute to durable disease resistance in the field but our understanding of PTI in many agriculturally important crop plants is only in its infancy. We have characterised the responses following recognition of the bacterial PAMPs elf18, flg22 and the fungal PAMP chitin in *Brassica napus* and *Brassica oleracea* . Assays have been developed to examine all stages of the *Brassica* PAMP response including early cell signalling events, such as the oxidative burst, MAPK phosphorylation and defence gene induction, to later stage cellular responses including callose deposition and lignification. Using these assays we discovered substantial variation existed in PAMP responses between *Brassica napus* varieties. We then mapped these responses within a *Brassica oleracea* double haploid mapping population and identified significant QTLs in the middle of chromosome 9. Interestingly, these QTLs localised to the same region as QTLs for susceptibility to *Agrobacterium tumefaciens* and *Botrytis cinerea* within the same cross. An approach combining next generation RNA sequencing and fine mapping is now being adopted to identify the candidate genes responsible for the substantial variation in PAMP responses, and pathogen resistance, within this population.

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Inception of infection: the boon and the curse for *Pseudomonas syringae*

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It has long been observed that environmental conditions play crucial roles in modulating immunity and disease in plants and animals. For instance, many bacterial plant disease outbreaks occur after periods of high humidity and rain. A critical step in bacterial infection is entry into the plant interior through wounds or natural openings, such as stomata. Recent studies have shown that stomatal closure is an integral part of the plant immune response to reduce pathogen invasion. In this study, we found that high humidity can effectively compromise stomatal immunity in both common bean and *Arabidopsis*, which is accompanied by down-regulation of the salicylic acid pathway and up-regulation on the jasmonic acid pathway. Specifically, bacterium-induced expression of *PR* genes is abolished, whereas several JA-responsive genes such as *JAZ, LOX3, OPR3, and AOC3*, are induced within a short period of time after transferring plants to high humidity. Highly humid environment can be conducive for plant infection by weak pathogens. On the other hand, periods of darkness, when most stomata are closed, are effective in decreasing pathogen penetration into leaves. However, coronatine produced by *Pseudomonas syringae* pv. *tomato* DC3000 cells can open dark-closed stomata facilitating infection. We conclude that: 1) a well-known disease-promoting environmental condition, high humidity, acts in part by suppressing stomatal immunity and 2) virulence factors, such as coronatine, appear to provide epidemiological advantages to ensure bacterial infection even when environmental conditions (darkness and insufficient humidity) favor stomatal immunity.

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Leucine derived hydroxy nitrile glucosides in barley and their relation to powdery mildew infection

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Barley is producing five hydroxy nitrile glucosides (HNGs). One of them, Epiheterodendrin, is a cyanogenic glucoside. Cyanogenic glucosides are well-known defence compounds distributed widely in the plant kingdom. They release poisonous hydrogen cyanide upon degradation by a specific β-glucosidase. However, no β-glucosidase is known to be present in barley leaves. More than 99% of the HNGs in the leaf are found in the epidermal cell layer. This may be subject to further subdivision, as we encounter HNGs in wax isolated from 1st leaves of one and two weeks old barley plants. The extracellular presence of HNGs is intriguing as well as surprising. We encounter the HNGs extracellularly when wax is sampled either with chloroform extraction or stripping the leaves with cellulose acetate or with gum Arabic. After applying the latter method the leaf does not wither. Further examination of the stripped leaves is performed visually as well as with scanning electron microscopy to ensure that the epidermal cell layer is intact after stripping. The presence of HNGs in the wax can serve many purposes, such as acting as a defence compound towards probing insects or fungi, as well as volatile breakdown products of HNGs could play a role in the plants communication with its surroundings. It is hypothesised that barley powdery mildew (*Blumeria graminis* sp. hordei) uses HNGs as food source or host recognition factor. This is further supported by the fact that bgh prefers high level HNG cultivars over low level HNG cultivars.

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RNA-seq analysis of the tomato immune response identifies genes whose expression is induced by MAMPs and suppressed by type III effectors

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Plants activate a variety of defense responses upon recognition of microbe-associated molecular patterns (MAMPs). This MAMP-triggered immunity (MTI) represents a first layer of defense and it can be suppressed by pathogens through the delivery of effectors into the cytoplasm. We used Illumina RNA-seq to perform a transcriptomic analysis of tomato leaves challenged with MAMPS (flg22, LPS, PGN), *Pseudomonas syringae* pv. *tomato* DC3000 and mutants (δ*hrcQ-U*, δ*hrcQ-U*/δ*fliC*, δ*28*, δ*avrPto*/δ*avrPtoB*), other *Pseudomonas* (*P. fluorescens*, *P. putida*) or *Agrobacterium tumefaciens*. An initial analysis of the treatments revealed the existence of two separate clusters: one that included mock, δ*hrcQ-U*/δ*fliC*, *A. tumefaciens*, LPS, PGN and DC3000, and the other containing the rest of the treatments. This distribution appears to be due to the perception or not of flagellin. Interestingly, wildtype DC3000, which expresses flagellin, clusters with the first group, indicating that the effectors delivered into the plant cell are largely involved in suppressing the transcriptional reprogramming that occurs upon flagellin perception. This large transcriptional suppression associated with delivery of the type III effectors is exemplified by the observation that of 2,600 genes induced by flg22, 1,600 are different when comparing DC3000 and the δ*28* "effector-less" mutant. We are currently focusing on this set of genes, especially those encoding protein kinases, whose expression appears to be targeted (directly or indirectly) by effectors, in order to identify novel genes involved in MTI.

PS13-479

Transcription factors, which connect MAMPs-responsive MAPK cascade to the coordinately-expressed phenylpropanoid biosynthesis genes

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MAPK cascades play an important role in induction of MAMPs-triggered immunity (MTI). We previously demonstrated that OsMKK4-OsMPK3/OsMPK6 constitutes a MTI signal cascade in rice. The activation of this MAPK cascade coordinately induces

the expression of 38 of phenylpropanoid biosynthesis genes. Therefore, we expected that common elements exists in the promoter regions of these phenylpropanoid biosynthesis genes. We searched such common elements and found a novel consensus sequence, designated MCA-box, in the promoter regions of 34 of phenylpropanoid biosynthesis genes. The MCA-box is similar to L-box, which is found in *PAL* promoter of dicotyledonous plants. In tobacco, L-box containing promoter is activated by R2R3-type MYB transcription factor (TF), NtMYB2. We found that active-OsMKK4 induces the expression of *OsMYB30, OsMYB55* and *OsMYB111*, which are orthologs of NtMYB2 in rice. Transient expression of these rice MYB cDNAs induces the expression of phenylpropanoid biosynthesis genes in rice calli. In addition, *OsMYB30* and *OsMYB55* genes are rapidly induced after MAMPs treatment. These data indicate that OsMYB30 and OsMYB55 are involved in the regulation of phenylpropanoid biosynthesis under MAMPs-response. Next, we analyzed how OsMKK4-OsMPK6 induces the *OsMYB55* expression. In tobacco, a GATA-type TF, AGP1, is a positive regulator of *NtMYB2*. We identified OsGATA3, a rice ortholog of AGP1, as a positive regulator of *OsMYB55*. Transient expression of OsGATA3 cDNA activates *OsMYB55* promoter. Further, addition of active-OsMKK4 and OsMPK6 enhanced the activity of OsGATA3. These results revealed a novel pathway in which OsMKK4-OsMPK6 activates OsGATA3, followed by promotion of *OsMYB55* expression to induce phenylpropanoid biosynthesis genes.

PS13-480

Isolation and characterization of lms, a rice lesion mimic mutant with enhanced resistance to rice blast (Magnaporthe oryzae)

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The lesion mimic and senescence (lms) mutant is characterized by a spontaneous lesion mimic phenotype during its vegetative growth and accelerated senescence after flowering. To isolate the OsLMS gene, mutant F2 plants obtained by crossing the lms mutant (japonica) to Kasalath (indica) were used to map the candidate region to about 322-kb on the long arm of chromosome 2. By Illumina whole-genome re-sequencing of the mutant, we identified a mutation causing a G to A nucleotide substitution at the exon-intron splicing junction of a gene encoding a protein with a carboxyl-terminal domain (CTD) phosphatase domain and two double stranded RNA binding motifs (dsRBM). The mutation causes a splicing error that is predicted to introduce a premature stop codon. RNA interference (RNAi) transgenic lines with suppressed expression of the LMS that display the lesion mimic phenotype confirmed that the mutation in LMS is responsible for the abnormal mutant phenotypes. A leaf blade spot inoculation test revealed lms shows enhanced resistance to a compatible race of rice blast compared to the wild-type plants. OsLMS shares a moderate amino-acid similarity to the *Arabidopsis FIERY2/CPL1* gene, which is known to control many plant processes such as stress response and development. The lms mutant also shows sensitivity to cold stress at the early growth stage, suggesting that LMS is a regulator of stress response in rice.

PS13-481

Functional characterization of *Arabidopsis WRKY55* gene in plant defense against a bacterial pathogen

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To identify the defense-related genes mediated by the MAPKK-MPK3/MPK6 cascade, genome-wide gene expression profiling was performed using the commercially available *Arabidopsis* Affymetrix microarray from NtMEK2^{DD} transgenic *Arabidopsis* plants after DEX treatment. Here, we describe that the roles of *AtWRKY55* gene, which is one of the genes regulated by MPK3/MPK6 cascade, involved in plant defense response. The expression of *AtWRKY55* genes was partially compromised in *NtMEK2^{DD}/mpk3* and *NtMEK2^{DD}/mpk6* plants. Expression of *AtWRKY55* was induced by pathogen infection and SA treatment. Both the T-DNA insertion mutants and overexpression transgenic lines were examined for responses to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000). Growth of bacterial pathogen was decreased in the overexpression transgenic lines. Futhermore, the AtWRKY55-overexpressing plants displayed enhanced expression of *PR-1* gene after *Pst* DC3000 infection. By contrast, T-DNA mutants showed enhanced growth of *Pst* DC3000 and suppressed expression of *PR-1* gene after bacterial infection. Taken together, these results suggest that AtWRKY55 has a positive role in plant resistance to bacterial pathogen.

PS13-482

A dual Resistance-protein system confers resistance against fungal and bacterial pathogens

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We found that both *Arabidopsis RPS4* and *RRS1* are required for resistance to *Colletotrichum higginsianum*, *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *tomato* strain DC3000 expressing *avrRps4*. These two adjacent *R* genes confer resistance to three distinct pathogens with very different infection strategies. Although the comparison of amino acid sequences of the *RPS4* alleles from twenty ecotypes revealed the amino acid sequences were highly similar, we found several variations in the LRR domain and C-terminal region of RRS1. Natural variation in receptor-type R proteins often occurs in their LRR domain, typically at the solvent exposed β-strand/β-turn structure. The strong selection pressure at the LRR domain suggests that this is the domain directly binds to the pathogen determinants that are evolving fast. To analyze the structure and function of RPS4 and RRS1 proteins, we introduced amino acid changes into RPS4 and RRS1. In this report, we will present the analysis of these mutants and the function of RPS4 and RRS1.

PS13-483

Breaking restricted taxonomic functionality by dual resistance genes

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In traditional breeding programs, introgression of disease resistance (*R*) genes from wild relatives into susceptible crops has been used for decades. However, transgenic transfer of NB-LRR type *R* genes between different plant families has not been successful, representing a phenomena called restricted taxonomic functionality. In previous study, we demonstrated that a pair of *Arabidopsis thaliana* TIR-NB-LRR genes *RRS1* and *RPS4* function together in disease resistance

against multiple pathogens, *i.e*., fungal pathogen *Colletotrichum higginsianum*, bacterial pathogens *Pseudomonas syringae* pv. *tomato* strain DC3000 expressing *avrRps4* (*Pst-avrRps4*) and *Ralstonia solanacearum*. We successfully transferred a genomic fragment containing *RRS1* and *RPS4* under control of the native promoter into the Brassicaceae, Solanaceae and Cucurbitaceae plants. The dual *R* gene transgenic plants were resistant to the fungal and bacterial pathogens. The transgenic plants did not have a stunted phenotype with spontaneous cell death. These results indicate that dual *R* gene can function between different families, breaking the dogma of restricted taxonomic functionality of *R* genes. This implies that the downstream components of *R* genes must be highly conserved and that interfamily utilization of *R* genes can be a powerful strategy to combat pathogens.

PS13-484

Microarray analysis of gene expression profiles induced by neutralized phosphorous acid and *Phytophthora parasitica* in tomato

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Phosphonate and related fungicides such as neutralized phosphorous acid (NPA) have been shown to be effective in controlling plant diseases caused by *Phytophthora*. In addition to its role as a fungicide, phosphonate has the ability to induce plant resistance against oomycete pathogens. To investigate the mechanism underlying phosphonate-induced resistance, we analyzed the transcriptomes of tomato, by using Tomato Genome Array (Affymetrix), in response to NPA treatment and NPA treatment followed by *P. parasitica* infection. The results showed that 91 genes were up-regulated (> 1.75 fold) and 20 genes were down-regulated (< 0.57) in response to NPA treatment. Functional classification of up-regulated genes by Gene Ontology (GO) analysis showed that most of the genes are in the category of "response to stress" and "cellular process". Noteworthy, they included genes involved in biotic stress resistance, such as jasmonic acid and ethylene signaling, polyamine biosynthesis, and chitin metabolic process. On the other hand, in comparison to water-pretreated control, 100 genes were induced and 59 genes were repressed specifically in NPA-pretreated tomato plants after *P. parasitica* inoculation. GO term analysis indicated that those up-regulated genes are in the category of "cellular process", "protein modification", and "response to stress". Interestingly, this group includes genes involved in "protein ubiquitination", "respiratory burst", and "response to chitin". Our results suggested that phosphonate confers plant resistance against oomycete pathogens through two distinct mechanisms: (1) accumulation of antimicrobial materials before encountering pathogen, and (2) rapid changes in oxidative burst and protein modification to regulated plant defense responses upon pathogen infection.

PS13-485

Molecular mechanisms for disease resistance in rice that is regulated by the transcriptional activator OsWRKY53

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OsWRKY53 is a transcriptional activator whose expression is enhanced in response to a chitin oligosaccharide elicitor from the rice blast fungus Magnaporthe oryzae. Overexpression of OsWRKY53 in rice induced up-regulation of defense-related genes, and resulted in enhanced resistance to M. oryzae, suggesting that OsWRKY53 plays important roles in elicitor-induced defense signaling pathways. We previously demonstrated that OsWRKY53 is phosphorylated in vitro by a MAP kinase cascade, and transactivation activity of phospho-mimic OsWRKY53 (W53PM), a constitutively active form, is higher than that of native OsWRKY53 (W53NT). In this study, we generated transgenic rice plants overexpressing W53PM and W53NT, and examined blast-resistance in parallel with transcriptome changes to M. oryzae infection in these plants. W53PM plants showed stronger resistance than W53NT plants and non-transformants (NTr). In the transcriptome analysis, 6842 genes were up-regulated in NTr plants 48 hours after blast-infection. We looked for genes expressed higher in only W53PM plants (group 1) or both W53PM and W53NT plants (group 2) in comparison with genes up-regulated in NTr plants. In W53PM plants, 175 genes were up-regulated at higher levels than in W53NT plants or NTr and 71 genes expressed higher in both W53NT and W53PM plants, suggesting that many genes have enhanced expression only in W53PM plants. Gene Ontology analysis revealed that 6% and 9% of genes in group 1 and 2, respectively, were categorized as defense-related genes. ChIP-seq analysis using W53NT-overexpressing cells enabled us to identify the target genes of OsWRKY53, whose regulation mechanisms by OsWRKY53 are of current interest.

PS13-486

Characterization of a novel pathogenesis-related protein from *Solanum lycopersicum*

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Phosphonate-based fungicides such as neutralized phosphorous acid (NPA) are known to induce plant resistance against many diseases, including those caused by *Phytophthora*. To investigate the mechanism underlying NPA-induced resistance, we previously performed a microarray analysis and found that a variety of defense genes were induced in response to NPA treatment in tomato plants. Among them, one gene (named NIPRa), which showed homology to a putative pathogenesis-related (PR) gene in barley, is significantly induced but functionally unknown. Hence, the aim of this study is to uncover the characteristics of NIPRa. Analysis by semi-quantitative reverse transcriptase-PCR indicated that expression of NIPRa was induced when plants were challenged with either *P. parasitica* or the bacteria wilt pathogen *Ralstonia solanacearum*. As well, NIPRa was up-regulated by salicylic acid and ethylene treatment. To test whether NIPRa contributes to plant resistance against pathogens, we overexpressed NIPRa by PVX agroinfection, and then challenged the plants with either *P. parasitica* or *R. solanacearum*. Plants overexpressing NIPRa showed higher tolerance to infection by these pathogens. In contrast, down-regulation of NIPRa by TRV-induced gene silencing increased plant susceptibility to pathogen infection. Furthermore, the NIPRa recombinant protein purified from *E. coli* showed green color and tended to form dimers to polymers when analyzed by gel filtration. Analysis by ICP-MS indicated that the recombinant NIPRa is most likely a metalloprotein. These results suggested that NIPRa may represent a novel category of PR protein, yet its function needs further investigation.

PS13-487

The transcriptional response in potato to infection by *Pectobacterium carotovorum* subsp. *brasiliensis* and the role of coronafacic acid in manipulating plant defences

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Pectobacterium carotovorum subsp. *brasiliensis* (*Pbr*) causes blackleg of potato stems and soft-rot of potato tubers. Genome sequencing of *Pbr* NZEC1, a highly virulent isolate collected from potato in New Zealand, identified the presence of a biosynthetic cluster encoding coronafacic acid (CFA). CFA was previously shown to be an important virulence factor in the bacterial potato pathogens *Pectobacterium atrosepticum* and *Streptomyces scabies*. Here, we show that inactivation of CFA significantly reduced the ability of *Pbr* NZEC1 to cause blackleg as well as soft rot, depending on the physiological age of the tubers. CFA is a component of coronatine, a phytotoxin involved in pathogenicity of the hemibiotrophic pathogen *Pseudomonas syringae* on numerous hosts. In *P. syringae*, coronatine functions as a molecular mimic of Jasmonic Acid (JA), resulting in up-regulation of genes related to JA signaling upon infection and subsequent suppression of the salicylic acid signaling pathway. To date, however, the influence of CFA on the host response to a necrotrophic pathogen such as *Pbr* remains unknown. In this study, Illumina-based RNA sequencing was used to compare the transcriptional response in potato tubers infected with either wild-type *Pbr* NZEC1 or a CFA- mutant. Analysis of the transcriptional data revealed that genes involved in both JA and ethylene biosynthesis were significantly differentially regulated in wild-type compared to the CFA- mutant, suggesting that CFA regulates both the pathways in tubers that lead to defensin production. Defensin is required for plant defence against necrotrophic pathogens.

PS13-488

Gene expression analysis during acibenzolar-S-methyl induced systemic disease resistance in cucumber using cross species microarrays

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In this study, expression was analyzed in order to identify genes of cucumber involved in systemic induction of resistance against anthracnose disease consequent to priming with the resistance inducer, acibenzolar-S-methyl (ASM). The first true leaves of cucumber plants were dipped in ASM suspensions at 100 mg/L a.i. or distilled water. Treated and untreated upper leaves were inoculated with *Colletotrichum orbiculare* 3 h after treatment. Samples were collected from third leaves at 24 h after inoculation. Leaf discs were soaked in RNA later® Tissue Collection until use for RNA isolation and cDNA synthesis. A custom (44k) Agilent microarrays comprising 29,756 probes were designed using the gene necleotide sequences from closely related and well annotated sequences of Arabidopsis, tobacco, cucumber etc. We identified 449 up-regulated and 378 down-regulated genes in ASM primed and pathogen inoculated cucumber plants, while ASM alone treated plants showed 133 up-regulated and 276-down regulated genes. These differentially regulated genes belonged to several hormonal pathways such as gibberellins (*GA2ox8*), abscisic acid (*ZEP*, *NECD1*), salicylic acid (*NPR1*), jasmonic acid (*LOX*, *AOC*) and ethylene (*ACS2*) pathways as well as chemical defense systems such as phenolics (*PAL*, *CAD* etc.) and lignification (*LPO*). The treatment with ASM alone provided significant pattern which will assist the concept of priming. The pattern of gene expression is in agreement with our previous studies and published literatures. In spite of requirement for validations, the concept of nearest neighbor microarray approach provides quick means for viewing global gene expression profiles without the dependability on transcriptome/ genomic sequence data.

PS13-489

Regulation of hypersensitive response by translationally controlled tumor protein in *Nicotiana benthamiana*

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Hypersensitive response (HR) is the most characteristic plant immune response. *Ralstonia solanacearum* (Rs8107) is non pathogenic and induces hypersensitive response in *Nicotiana benthamiana*. We have isolated and analyzed genes, which are regulated by inoculation with *R. solanacearum* in *N. benthamiana* plants (*R. solanacearum*-responsive genes; RsRGs) related to the HR by virus-induced gene silencing using *Nicotiana benthamiana* and the *Potato virus X* vector system. We selected a RsRG308, since an HR induction was accelerated in RsRG308-silenced plants challenged with Rs8107. Deduced amino acid sequence of full length RsRG308 cDNA showed similarity to translationally controlled tumor protein (TCTP) genes from *Arabidopsis thaliana*, *Glycine max*, *Oryzia sativa*, *Triticum astivum*. Then, we designated the cDNA as *NbTCTP* (*N. benthamiana* translationally controlled tumor protein). Acceleration of HR cell death and over-production of reactive oxygen (ROS) were observed in *NbTCTP*-silenced plants inoculated with Rs8107, *Pseudomonas syringae pv. syringae* and *P. chichorii*. Acceleration of HR cell death was also observed in *NbTCTP*-silenced plants by *Agrobacterium tumefaciens*-mediated transient expression of HR elicitors and a constitutively active form of mitogen-activated protein kinase kinase. The bacterial population of Rs8107 was reduced in *NbTCTP*-silenced plants compared to control plants. These results suggested that *NbTCTP* might have regulatory function in HR cell death via reactive oxygen mediated signaling pathway.

PS13-490

NOD1, a negative regulator of plant immune response, is required for establishment of disease susceptibility during *Nicotiana benthamiana-Ralstonia solanacearum* interaction

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Plants have evolved an immune system to reject microbial infections. However, adapted microbes (pathogens) suppress the immune system and induce disease susceptibility by targeting host molecules that function as negative regulators of immune responses. In order to better understand the molecular mechanisms of disease susceptibility, we have used the *Nicotiana benthamiana-Ralstonia solanacearum* pathosystem. Virus-induced gene silencing was used to screen for the plants that failed to develop wilt symptom in response to *R. solanacearum*. Among the screened plants, we focused on the plant showing a highly resistance phenotype against *R. solanacearum*, and designated as NOD1 (No disease 1) plant. Silencing of *NOD1* resulted in a dramatic increase of reactive oxygen species (ROS), and overproduction of ROS in NOD1 plants was reduced by double silencing of *NOD1* and *NbrbohB*. The wilt symptom was observed in *NOD1/NbrbohB*-silenced plants similar to control plants, indicating involvement of ROS signaling in the resistance of NOD1 plants. Intriguingly, *NOD1* gene was drastically expressed in leaves inoculated with a wild type of *R. solanacearum*, but not with a type 3 secretion system (T3SS)-deficient mutant of the bacteria. Taken together, NOD1 may act as a negative regulator of plant immune responses, and be targeted by bacterial effector(s) during the establishment of disease susceptibility.

PS13-491

A positive regulatory role of the watermelon *ClWRKY70* gene for disease resistance in transgenic *Arabidopsis*

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A pathogen-inducible WRKY cDNA was cloned from the leaves of watermelon seedlings 24 h after inoculation with *Cladosporium cucumerinum*. The deduced protein of the gene, designated as ClWRKY70, was classified as a group III WRKY protein based on its single WRKY domain containing a Cys2HisCys zinc-finger motif. Its *Arabidopsis* sequence homologue (AtWRKY70) has been described as playing an important role in the plant defense response. *ClWRKY70* gene transcripts were highly accumulated in watermelon by salicylic acid treatment, but not by jasmonic acid. By evaluating target gene expression in transgenic *Arabidopsis* overexpressing the *ClWRKY70* gene, it is suggested that the watermelon WRKY gene may play a positive regulatory role in plant resistance against pathogen attack.

PS13-492

The Arabidopsis anion channels participate in innate immunity

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The anion efflux is one of the early responses of plant cells to pathogen attacks. However, which and how anion channel participates in the process is still unknown. In animal, it was found that anion channel is involved in the programmed cell death. In plants, it is also reported that anion efflux regulates hypersentive response (HR) cell death. We are aiming to identify the anion channels involved in plant defense responses. We systematically studied the Arabidopsis anion channel families by isolation of T-DNA insertion lines, disease resistance assays and electrophysiology and Cl-sensor analyses of anion flux in host cells. Preliminary data showed that Arabidopsis support that different anion channels may play both negative and positive roles in PAMP-triggered immunity (PTI) and effector-triggered immunity.

PS13-493

High-throughput screening of chili pepper proteases function in *Nicotiana benthamiana* following pathogens infections

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Plant genome encodes hundreds of proteases, which break peptide bond of proteins. Proteases play key role in regulation of biological processes in plants which include plant metabolism, physiology, growth and defense. Proteases are classified into 5 families: Cystein proteases, Serine proteases, Threonine proteases, Metallo proteases and Aspartic proteases based on the nucleophile and oxyanion stabilizer. We have selected 940 putative proteases from EST of *Capsicum annuum* using protease domain from MEROPS database through blastX, hmmpfam and hmmsmart. To identify novel functions of pepper proteases, we have cloned 159 proteases into TRV-LIC vector to perform virus-induced gene silencing. As a gene silencing results, 29 proteases-silenced phenotypes showed growth retardation, 9 showed severe stunting with crinkled leaves, 8 showed severe stunting, 7 showed crinkled leaves and lethality, 4 showed variegated leaves, 3 showed yellowing leaves, 95 showed no difference and the other 4 showed various phenotypes. These results may indicate that plant proteases have essential roles in plant growth and development. Currently we are working on the roles of plant protease in pathogen defense. To identify the role of proteases in pathogen defense, we have infected avirulent and virulent pathogen to the protease-silenced plant. As pathogen infection results, 12 showed enhanced HR, 31 showed delayed HR and 14 showed delayed disease symptom. Progresses of our work on functional genomics of chili pepper protease gene superfamily will be presented as poster.

PS13-494

***Capsicum*-specific secreted protein CaSD1 has multiple roles in pathogen defense, delay of senescence, and trichome formation**

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Secreted proteins have diverse functions involving in plant development, metabolism, and defense response against pathogens. *Capsicum annuum senescence-delaying 1* (*CaSD1*), a gene encoding a novel secreted protein, was isolated from peppers (*C. annuum* CM334) using the yeast secretion trap system following inoculation with *Phytophthora capsici*. CaSD1 is present only in species of the *Capsicum* genus and contains multiple repeat sequences of "KPPIHNHKPTDYDRS". Interestingly, the number of repeat units was variable among species and cultivars in the *Capsicum* genus. *CaSD1* is expressed in roots at normal condition, but the transcript levels of *CaSD1* were rapidly upregulated in leaves when treated with either pathogens or defense-related signaling molecules. *Agrobacterium*-mediated transient overexpression of CaSD1 in *Nicotiana benthamiana* resulted in delayed senescence with a dramatically increased number of trichomes and enlarged cell size. Furthermore, certain senescence- and cell division-related genes were differentially regulated by CaSD1-overexpressing plants. These observations imply that the pepper-specific cell wall protein CaSD1 might have roles in plant growth and development as well as in pathogen defense.

PS13-495

Development of a high-throughput system to monitor pathogen-responsive gene expression in *Arabidopsis thaliana* seedlings using bioluminescent reporters

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To develop a bioluminescence monitoring of plant defense gene expression, we exploited firefly luciferase (Fluc) reporter system and tested several defense-related gene promoters using transgenic Arabidopsis. Results of *in vivo* bioluminescence assay indicated that the promoters are induced in response to treatment with chemicals or pathogen inoculation and the luminescence levels are in parallel with the endogenous mRNA levels. In order to adapt to the high throughput screening (HTS) system in 96-multiwell format, we further selected promoters that are functional in growth stage 1.0 seedlings (cotyledon fully opened), and found that the *Pathogenesis Related protein 1a* (*PR-1a*) from tobacco BY-2 and the *Vegetative Storage Protein 1* (*VSP1*) promoter from *Arabidopsis thaliana*, showed clear Fluc activity induction in response to treatment with chemicals in *A. thaliana* seedlings. Using this technology, we could successfully identify chemicals with defense gene inducer activity that can be applicable to the development of plant activators from the chemical libraries. Also, we obtained mutants with altered defense gene expression from the populations of EMS-mutagenized M2 seedlings. To improve the system to overcome problems, we are currently introducing the dual-color luciferase assay system using click beetle luciferases from *Pyrophorus plagiophthalamus* as reporter genes.

PS13-496

The influence of infection pressure of *Synchytrium endobioticum* (Schilb.) Perc. on reaction of potato

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Synchytrium endobioticum is a soil-borne quarantine pathogen of potato caused Potato Wart Disease (PWD). There is no chemical control of PWD and the only natural resistance of the potato is the way of elimination this pathogen. The most varieties of potato are resistant or susceptible to pathotype 1(D1) of *S. endobioticum*. Some varieties are partially resistant. They react as resistant in low infection pressure and susceptible in high infection pressure of *S. endobioticum*. Four varieties (Erika, Signum, Bonus and Allora) of potato were tested using two different method of inoculation: Spieckermann method with winter sporangia (low pressure of pathogen) and Glynne-Lemmerzahl method with summer sporangia (high pressure of pathogen). Pathotype 1(D1) of *S. endobioticum* was used in both methods. Although inoculum was characterized (40000 winter spores or 2 g of fresh wart / tuber), there is no possible to direct control number of zoospores during infection. In the case of winter sporangia only a few are capable of germination during inoculation period (6-8 weeks) in contrary to summer sporangia when the most of sori release zoospores in the same time (2 days). All varieties were resistant after using winter sporangia while using of summer sporangia broke down partially resistant of varieties leading to close the live cycle of *S. endobioticum*. The presence of winter sporangia in host's tissue of varieties were a proof for susceptibility. Only high infection pressure of *S. endobioticum* allows for adequate reaction of host and distinguish truly resistant varieties of potato among partially resistant ones.

PS13-497

Suppression of autophagosome formation by cryptogein, a proteinaceous elicitor from an oomycete, in tobacco BY-2 cells

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Possible involvement of autophagy in immune responses has extensively been discussed both in plants and animals. However, little is known on the dynamics of autophagy during the induction of defense responses in plant cells. Cryptogein, a protein from an oomycete, elicits a series of defense responses including reorganization of the vacuoles and hypersensitive cell death in tobacco BY-2 cells (Higaki et al. 2007). We here developed an *in vivo* imaging system to monitor the dynamics of autophagy in BY-2 cells expressing YFP-NtAtg8. The number of autophagosomes rapidly decrease within 15 min in response to cryptogein. Not only initial defense responses including NADPH oxidase-mediated ROS production but also suppression of autophagosome formation triggered by cryptogein required continuous recognition of the elicitor, and severely inhibited by a protein kinase inhibitor, K-252a. Possible physiological and pathological significance as well as the regulation of autophagy during the induction of plant immune responses will be discussed.

PS13-498

Roles of an S-type anion channel SLAC1 in the regulation of cryptogein-induced initial responses and hypersensitive cell death in tobacco BY-2 cells

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Depolarization and anion effluxes through the plasma membrane are often rapidly induced during defense responses in plant cells (Kuchitsu et al. 1993, 1997). Pharmacological analyses suggest their essential roles for the induction of innate immunity and hypersensitive cell death (Kadota et al. 2004). However, the molecular bases for the anion effluxes and their regulation in immune responses remain largely unknown. SLAC1 (SLOW ANION CHANNEL-ASSOCIATED 1) has recently been identified as a plasma membrane slow-type (S-type) anion channel in *Arabidopsis* stomatal guard cells (Negi et al. 2008; Vahisalu et al. 2008). We here overexpressed *Arabidopsis SLAC1* gene in tobacco BY-2 cells and investigated its effects on cryptogein-induced initial responses including various ion fluxes and NADPH oxidase-mediated ROS production as well as downstream events such as expression of defense-related genes and hypersensitive cell death. The SLAC1-GFP fusion protein was localized at the plasma membrane. The overexpressors showed enhanced sensitivity to cryptogein to induce a wide range of immune responses, which were suppressed by an S-type anion channel inhibitor. Possible roles of SLAC family anion channels in plant immunity will be discussed.

PS13-499

UV-B irradiation-induced suppression of necrotic symptom development and TSWV accumulation in tobacco plants

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Ultraviolet-B (UV-B, 280-320 nm) irradiation triggers stress responses accompanied by changes in the expression of a large number of genes in plants. The UV-B-responsive genes include those involved in disease resistance; therefore, UV-B irradiation has been thought to enhance resistance to pathogens. However, there is little experimental evidence regarding the effect of UV-B on disease resistance. Thrips-transmitted *Tomato spotted wilt virus* (TSWV) causes an important disease in a wide range of plants. Here, we report that UV-B irradiation reduces the incidence of disease caused by TSWV in tobacco. In tobacco plants, TSWV spreads systemically and triggers the development of necrotic lesions. Exposure of the tobacco plants to UV-B irradiation after TSWV inoculation suppressed necrotic symptom development and TSWV accumulation in an intensity-dependent manner. Pretreatment of the tobacco plants with UV-B irradiation before inoculation also produced the same inhibitory effect. However, TSWV inoculum exposed to UV-B irradiation exhibits the same level of infectability as the non-irradiated control. These results suggest that the suppression is attributable to the UV-B-induced defense responses in the host plants. We also report a difference in gene expression on TSWV infection between UV-B irradiated and non-irradiated plants.

PS13-500

Cloning and expression analysis of an arginine decarboxylase gene from bottle gourd (*Lagenaria siceraria*)

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Bottle gourd has been used as a source of rootstock for watermelon against soil-borne disease and low soil temperature. However, the wilt in cucurbit crops caused by abiotic stress such as drought has

been reported recently. Genetically modified plant breeding could be used to improve stress tolerance of bottle gourd rootstock. Therefore, we tried to clone an arginine decarboxylase (ADC) gene, which is involved in plant putrescine (Put) biosynthesis, from bottle gourd and to analyze its expression in abiotic stress conditions. The full length of *LsADC* gene was isolated through RT-PCR using primers designed based on highly conserved region of cucumber ADC gene. Sequence analysis by BLASTX program revealed that the putative amino acid sequence of *LsADC* shared high identities with known ADCs from other plants, such as cucumber (96%), *Nicotiana tabacum* (76%), *Arabidopsis thaliana* (71%) and rice (64%). The *LsADC* contained two well-conserved motifs characteristic of decarboxylase and a potential chloroplast transit peptide in the N-terminal. *LsADC* was expressed at high level in the stem, cotyledon and root, whereas a weak signal could be detected in the leaves. Transcripts of *LsADC* in bottle gourd leaves were induced continuously in response to drought and high salt treatment and pathogen infection as well. Taken together, *LsADC* is a stress-responsive gene and could be used as a candidate gene for bottle gourd genetic transformation in the future.

PS13-501

A novel communication between plants and soil bacteria through volatile substances

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Recent studies demonstrate that, while vast majority of soil bacteria do not exhibit significant effect on plant growth, selected strains of bacteria are capable of either promoting or inhibiting the growth of plants through volatile substances. However, to our knowledge, bacterial volatile substances that are responsible for this type of plant-microbe interaction have not fully identified so far, and more over, little is known about how plants sense and respond to the corresponding bacterial molecules. We report here that *Bacillus subtilis* strains tested in our study can inhibit the growth of various plant species including *Arabidopsis*, rice and basil seedlings without direct contact. In addition, the plant seedlings also exhibit similar growth retardation upon exposure to the volatiles of *Agrobacterium tumefaciens*. These results suggest that the plant's response to bacterial volatiles might be regulated by a mechanism that is broadly conserved among plant kingdom. Bioassay-based purification of bacterial metabolites that are responsible for the plant-bacteria interaction led us to obtain a fraction enriched in selected classes of metabolites. Putative biological roles of the bacterial volatiles will be also discussed.

PS13-502

Pathogen-induced ERF68 in tomato modulate production of reactive oxygen species that cause cell death and pathogen resistance

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Ethylene response factors (ERFs) are a large plant-specific transcription factor family and integrate external and endogenous signals and control plant growth, development and defense responses. Bacterial wilt (BW) caused by *Ralstonia solanacearum* is a world-widely serious and complex disease, causing significant crop losses; however, information on plant defense response to BW is limited. Our previous study suggested the involvement of thermo-stress responsive factor1 (TSRF1), a member of tomato ERF Group IX (SlERF-IX), in defense against BW. This study aimed to elucidate roles of additional ERF-IX members in defense response to BW. Six uncharacterized SlERF-IX members were identified in the tomato database. GFP-fused ERFs localized in the nucleus of Arabidopsis protoplast and transactivation assay confirmed these proteins are functional transcriptional activators. Interestingly, using virus-mediated gene overexpression (VMGO) approach, we found overexpression of *SlERF68* induced lesions in tomato leaflets. Similar phenotype was consistently observed in *Nicotiana benthamiana* and *N. tabacum* transiently overexpressing *SlERF68* by Agro-infiltration. *SlERF68* was abundantly expressed in root and flower bud, and its expression in leaves could be induced by salicylic acid and ethylene. In addition, *SlERF68* expression was highly induced by the wild-type *R. solanacearum* strain, but not by a mutant strain defective in the Type III secretion system, suggesting its involvement in effector-triggered immunity (ETI) response. Using an inducible transient expression system, our results showed that *SlERF68* overexpression triggered reactive oxygen species (ROS) accumulation and led to cell death. These results together reveal function of *SlERF68* in regulating ROS production in plant defense response against pathogen invasion.

PS13-503

Regulation of elicitor-induced Ca^{2+} influx and phytoalexin production by a voltage-gated Ca^{2+} permeable channel OsTPC1 in rice

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Various types of microbe- or plant-derived signaling molecules (MAMPs/DAMPs) or elicitors induce various temporal patterns of changes in the cytosolic concentration of free Ca^{2+} prior to a series of defense responses including biosynthesis of antimicrobial secondary metabolites called phytoalexins; however, the molecular links and regulatory mechanisms of the phytoalexin biosynthesis remains largely unknown. A fungal xylanase protein (TvX) induces defense responses including hypersensitive cell death in suspension-cultured rice cells. TvX induced a prolonged increase in cytosolic Ca^{2+}, mainly due to a Ca^{2+} influx through the plasma membrane. Membrane fractionation by two-phase partitioning and immunoblot analyses revealed that OsTPC1 is localized predominantly at the plasma membrane. In retrotransposon-insertional *Ostpc1* knock-out cell lines harboring a Ca^{2+}-sensitive photoprotein, aequorin, TvX-induced Ca^{2+} elevation was significantly impaired, which was restored by expression of OsTPC1. TvX-induced production of major diterpenoid phytoalexins and the expression of a series of diterpene cyclase genes involved in phytoalexin biosynthesis as well as hypersensitive cell death were also impaired in the *Ostpc1* cells. Whole cell patch clamp analyses of OsTPC1 heterologously expressed in HEK293T cells showed its voltage-dependent Ca^{2+}-permeability. These results suggest that *Ostpc1* plays a crucial role in TvX-induced Ca^{2+} influx as a plasma membrane Ca^{2+}-permeable channel consequently required for the regulation of phytoalexin biosynthesis and hypersensitive cell death in cultured rice cells. Recent advances in downstream Ca^{2+}-mediated signaling network involving NADPH oxidase-mediated production of reactive oxygen species will also be discussed.

PS13-504

HSP70 regulates Tabtoxinine-β-lactam-induced cell death in *Nicotiana benthamiana*

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Puseudomonas syringae pv. *tabaci* causes wildfire disease to *Nicotiana benthamiana*. This disease symptom is promoted by

Tabtoxinine-β-lactam (TβL), host non-specific bacterial toxin. TβL is known to inhibit glutamine synthetase, and induce plant cell death through the abnormally accumulated ammonia. However, the detail mechanisms of TβL-induced cell death have been obscured. In this study, we focused on *SGT1*, *RAR1*, *HSP90* and *HSP70*, which regulate various types of plant cell death in *N. benthamiana*. To analyze the roles of *SGT1*, *RAR1*, *HSP90* and *HSP70* on TβL-induced cell death, we carried out virus-induced gene silencing with *N. benthamiana* and *Potato virus X* vector systems, and the leaves of silenced and control plants were infiltrated with purified TβL. TβL-induced cell death was observed in *SGT1*-, *RAR1*- and *HSP90*-silenced plants similarly to control plants. In contrast, TβL-induced cell death was drastically suppressed in *HSP70*-silenced plants. In the *HSP70*-silenced plants treated with TβL, amount of ammonia was higher than that in control plants. Furthermore, the silencing of *HSP70* also suppressed cell death induced by treatment with L-methionine sulfoximine (MSX) that inhibit glutamine synthetase similarly to TβL. Over accumulation of ammonia was also observed in *HSP70*-silenced plants after treatment with MSX. These results suggested that HSP70 might be essential in TβL- and MSX-mediated cell death induction pathway, and have a role in downstream of excess ammonia accumulation.

PS13-505

Photosynthesis-mediated activation of PAMP-induced biosynthesis of salicylic acid in *Arabidopsis*

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Agronomic crop diseases occasionally result from insufficient sunlight, suggesting that light might be required for activation of plant defense responses against pathogen attack. However, the role of light in plant innate immunity remains largely elusive. Recently, we revealed that chloroplasts are involved in both PAMP-induced basal resistance and R gene-mediated hypersensitive cell death. Salicylic acid (SA) is a key regulator of plant defenses, which accumulates in in response to a variety of biotic stresses, including pathogen associated molecular patters (PAMPs). The pathways and regulation of SA biosynthesis in plants may be more complicated than previously thought. Here, we demonstrate that light is required for PAMP-induced biosynthesis of SA and subsequent activation of defense responses. Interestingly, we found that photosystem II inhibitor DCMU severely reduced PAMP-induced SA accumulation in the light, suggesting dependence of SA biosynthesis on a photosynthetic electron transport activity. Furthermore, PAMP-induced expression of SA biosynthesis genes is largely dependent on light and suppressed by DCMU. The present study reveals a previously unknown photosynthesis-dependent signaling pathway linking photosynthesis to PAMP-induced immunity including SA biosynthesis and subsequent defense responses.

PS13-506

Molecular analysis of rice *heme activator protein* (*OsHAP2E*) and *aspartic protease* (*OsAP77*) genes in response to biotic and abiotic stresses

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The expression of *rice heme activator protein* (*OsHAP2E*) and *aspartic protease*) (*OsAP77*) genes was induced by probenazole (PBZ), a chemical inducer of disease resistance. To elucidate roles of these genes, the chimeric genes (*OsHAP::GUS* and *OsAP::GUS*) have been constructed to carry the structural gene encoding &beta-glucuronidase (GUS) driven by the promoters from *OsHAP2E* and *OsAP77*, respectively. These constructs were introduced into rice. Transgenic lines were tested for GUS staining. Only the wound and surrounding tissues were stained blue for *OsAP::GUS* but not for *OsHAP::GUS*. However, when the chimeric gene (*OsHAPin::GUS*) was constructed to carry the *OsHAP77* promoter and its first intron, the transgenic lines of *OsHAPin::GUS* showed high GUS activity in the wound and surrounding tissues. Thus these promoters responded to wounding. The transgenic lines were further examined under abiotic and biotic stress conditions. When immersed in a solution containing salicylic acid, isonicotinic acid, abscisic acid or hydrogen peroxide, the GUS activity was observed exclusively in vascular tissues for *OsAP::GUS*, but in vascular tissues and mesophyll cells for *OsHAPin::GUS*. When inoculated with *Magneporthe oryzae* or *Xanthomonas oryzae pv. oryzae*, the transgenic lines showed the GUS activites in the area surrounding the necrotic lesions induced by the infection. These results suggest that the expression of these genes is induced by abiotic and biotic stresses.

PS13-507

Tryptophan-derived metabolites in the immunity of Brassicaceae species

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One of the evolutionary conserved responses of flowering plants to pathogen attack involves biosynthesis and secretion of secondary metabolites. Model plant *Arabidopsis thaliana* accumulates upon infection tryptophan derived indole-type metabolites including indole-3-carboxylic acids, phytoalexin camalexin and indole glucosinolates (IGs) with their downstream metabolism products. In this study we investigate the conservation and diversification of the pathogen-inducible tryptophan-derived metabolism in close and distant *A. thaliana* relatives by metabolic profiling. We substantiate the observed species-specific metabolic patterns by the presence or absence of candidate ortholog genes encoding enzymes involved in tryptophan metabolism in accessible genomes of *A. thaliana* relatives. Our metabolic survey reveals a surprising conservation of the pathogen-triggered IG metabolic and secretory pathway between the tested plant species, suggesting an ancient and important function of this metabolic branch in Brassicaceae pre-invasive defence responses. In contrast, I3CA and camalexin biosyntheses appear to be clade-specific innovations within the conserved framework of pathogen-inducible tryptophan metabolism and represent relatively recent manifestations of the plant-pathogen arms race.

PS13-508

Salicylic acid induces genes for the unfolded protein response depending on IRE1 and bZIP60 in *Arabidopsis*

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Salicylic acid (SA) is a phytohormone involved in signaling of systemic acquired resistance. SA has been also reported to induce genes related to the unfolded protein response (UPR) that is a cellular response highly conserved among eukaryotic cells to prevent abnormal maturation of proteins in the endoplasmic reticulum (ER). Induction of UPR genes such as ER resident molecular chaperones by SA has been considered to be regulated by a molecular mechanism different from that of the UPR. Recently, it was found that an ER membrane-localized sensor IRE1 catalyzes

cytoplasmic splicing of mRNA encoding a transcription factor bZIP60 in Arabidopsis. As a result of this splicing, an active form of bZIP60 is translated and enhances expression of UPR genes. More specifically, Arabidopsis has two IRE1 paralogs IRE1A and IRE1B with redundant function in splicing of *bZIP60* mRNA. In present study, we tried to clarify whether activation of bZIP60 by IRE1 is involved in the induction of the UPR genes by SA treatment. Genes such as *BiP3* and *Sar1* highly regulated by IRE1 and bZIP60 were induced by SA treatment (0.5 mM). Splicing of *bZIP60* mRNA was observed under this treatment. Induction of *BiP3* and *Sar1* was suppressed in T-DNA insertion mutants of *bZIP60* and *IRE1A/IRE1B*, but not in *npr1-1* lacking part of SA signaling pathway. Thus, induction of these UPR genes by SA was considered to be regulated by IRE1 and bZIP60, but not by NPR1.

PS13-509

Toward understanding of spatial and temporal regulation of hypersensitive response upon R-Avr recognition

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Plants possess several layers of defense against pathogens. The strongest immune system is controlled by Resistance (R) proteins, plant immune receptors. Specific recognition of a pathogen effector protein (known as Avirulence (Avr) protein) by a cognate R protein results in a rapid plant immune response, so-called hypersensitive response (HR), involving localized programmed cell death and accumulation of reactive oxygen species, salicylic acid and antimicrobial compounds, such as pathogenesis-related (PR) proteins and phytoalexins. Although HR is triggered by a specific R-Avr recognition, it still remains unclear whether all the responses constituting HR occur sequentially in a cell or differentially in different cells. In order to understand spatial and temporal regulation of HR, various transgenic Arabidopsis plants carrying different promoter-reporter constructs for plant defense-related marker genes have been generated. The use of fluorescent proteins, as well as an inducible Avr expression system, should provide a better resolution for dissecting temporal and spatial control of the HR. Current progress in this study will be discussed.

PS13-510

Two histone-modifying proteins regulate plant immune response against *Pseudomonas* infection

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In plants, recognition of invading pathogenic microorganisms by pattern recognition receptor and race-specific resistance protein activates diverse cellular responses to defend themselves against pathogen infection. One of well-known immune responses is the transcriptional reprogramming occurring when pathogen infects plant. The transcriptional reprogramming also takes place in priming of defense response that makes plant rapidly and strongly expresses defense-related genes against secondary pathogen infection. Chromatin remodeling caused by change of histone marks and replacement of histone variants affects gene expression that is important for either development or disease resistance. Arabidopsis mutants corresponding histone acetyltransferase, histone deacetylase, histone methyltransferase, histone demethylase and core histone proteins were collected to identify either *immune-defective* or *enhanced-immune* mutants. Here we reported two novel *enhanced-immune* mutants showing disease resistance against the pathogen *Pseudomonas syringae* pv. *maculicola*. The enhanced disease response only limited the growth of virulence pathogen. One of mutant, but not the other, accumulated higher level of salicylic acid than wild-type plants. Also the histone-modifying protein seemed to be physically interacted with a series of proteins. We will discuss the molecular function of the histone modifying genes in plant immunity. These works are supported by National Research Foundation of Korea-Excellent scientists from local universities and Rural Development Administration-Woo Jang Choon Project.

PS13-511

Role of ceramidases in *Arabidopsis* morphogenesis and disease resistance

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Sphingolipids are the prime lipid components of eukaryotic membranes and function through their metabolites as bioactive regulators of many cellular processes. Ceramidases, hydrolyze ceramide to long chain base, are key regulators in sphingolipids homeostasis. Little is known about the roles of ceramidases in plants. Here we report analysis of a homozygous T-DNA insertion ceramidase mutant *cds-1* (a homolog of yeast YPC1P/YDC1P). The *cds-1* mutants exhibited smaller, narrower and slightly greener leaves than the wild type leaves. We found that *cds-1* plants were more susceptible to bacterial pathogens and mycotoxin Fumonisin B1. Analysis of the patterns of GUS expression in *proAtACER*::GUS indicated that the ceramidase was highly expressed in pollen grains and leaf primordium. Using quantitative sphingolipid profiling, a high ceramide level was found in FB1 treated *cds-1* plants when compared with wild type plants. The possibility role of ceramidases in plant development and disease resistance will be discussed.

PS13-512

Several EAR motif-containing ERFs in group VIII are involved in HR cell death induction

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Ethylene response factors (ERFs) conform one of the largest transcription factors' families in plant. Recently, many *ERF* genes in various plant species have been reported to be involved in the responses to environmental stresses, both biotic and abiotic. Previously, we reported that transient overexpression of *NtERF3* gene induced hypersensitive response (HR)-like cell death in tobacco leaves. The ERF-associated amphiphilic repression (EAR) motif in the C-terminal region of NtERF3 was essential for its cell death-inducing ability. In *NtERF3*-silenced *Tobacco mosaic virus* (TMV)-resistant tobacco, lesion formation by TMV infection had a tendency to expand than that in non-transgenic tobacco (Ogata et al, J Gen Plant Pathol (2012) 78:8-17). NtERF3 is classified into group VIII when the ERF family proteins are divided based on the homology of the AP2/ERF DNA-binding domain. We isolated several EAR motif-encoding *ERF* genes in group VIII from tobacco, *Arabidopsis* and rice that harbored cell death-inducing ability. Some of the cloned tobacco genes were upregulated during HR induction by *N* gene-TMV interaction while others were not induced. Co-expression of a dominant-negative form of *NtERF3* suppressed the cell death induced by some other *ERFs* in group VIII. These results suggest that the deletion mutant of *NtERF3* operated in a dominant-negative manner to group VIII *ERFs* and that there are common target genes responsible for cell death induction to these *ERFs*. Using the dominant-negative form of *NtERF3*, we also show an evidence that *NtERF3* is regulated downstream of *NtSIPK* and *NtWRKY1*.

PS13-513

Bacteria induce systemic acquired resistance in barley

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This work shows for the first time that systemic disease resistance can be induced by a localized infection of barley. Until now, it has been unclear if systemic acquired resistance (SAR) exists in monocotyledonous plant species and/or how it compares to well-established SAR systems in dicotyledonous plants. Salicylic acid (SA), the key hormone for SAR in dicotyledonous plants, appears to play a role in disease resistance in, for instance, rice. In addition, SA signalling partners such as NPR1 are conserved in various monocotyledonous plants. We investigated the possibility to induce SAR in barley in order to generate a monocotyledonous SAR pathosystem to test possible protection of cereals via SAR/priming. Infection of the first leaf of 4-week-old barley plants with either *P. syringae* pathovar *japonica* or *Xanthomonas translucens* significantly enhanced resistance in the systemic tissue against *X. translucens*. *P. syringae* grew, but only to limited levels in the inoculated leaf and caused brown spots reminiscent of HR lesions, whereas *X. translucens* seemed virulent, growing to high levels within the leaves causing severe yellowing and eventually death. Both primary inoculi caused increased accumulation of SA and glucosylated SA in the infected leaf, but not systemically. We have performed micro array analyses of the infected and systemic tissue to investigate which genes are induced and/or repressed during systemic resistance induction in barley. Results of these analyses should reveal which disease resistance pathways are involved in the induced resistance response.

PS13-514

The transcriptome of *Verticillium dahliae*-infected *Nicotiana benthamiana* determined by deep RNA sequencing

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Verticillium wilts are diseases caused by fungi of the *Verticillium* genus that occur on a wide range of host plants, including Solanaceous species such as tomato and tobacco. Currently, the well characterized *Ve1* gene of tomato is the only *Verticillium* wilt resistance gene cloned. During identification of the *Verticillium* molecule that activates Ve1 resistance in tomato, RNA sequencing (RNA-seq) of *Verticillium*-infected *Nicotiana benthamiana* was performed. In total, over 99% of the obtained reads were derived from *N. benthamiana*. Here, we report the assembly and annotation of the *N. benthamiana* transcriptome. In total, 142,738 transcripts >100 bp were obtained, amounting to a total transcriptome size of 38.7 Mbp, which is comparable to the Arabidopsis transcriptome. About 30,282 transcripts could be annotated based on homology to Arabidopsis genes. By assembly of the *N. benthamiana* transcriptome, we provide a catalogue of transcripts of a Solanaceous model plant under pathogen stress.

PS13-515

The bilateral role of light in plant-pathogen interaction

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Plants have evolved sophisticated defense mechanisms against various stresses like diseases caused by pathogens. However, also pathogens have learned to respond to and evade the defense mechanisms of plants and this complex arms race is further affected by abiotic environmental factors such as light. Light plays a central role in plant-pathogen interaction as many plant defense responses are light-dependent. Intriguingly, since light also affects pathogen growth and virulence, we aim to characterize this bilateral role of light in more detail and thus, expand the big picture of plant-pathogen interactions. Arabidopsis thaliana gene Early Responsive to Dehydration 15 (ERD15) is rapidly induced in response to various environmental factors including pathogens. Plants overexpressing ERD15 are insensitive to ABA resulting in e.g. impaired stomatal closure, but also, improved pathogen tolerance. Furthermore, localization studies place this protein to chloroplast, which is an important organelle in plant light adaptation. Interestingly, light is also one of the triggers for stomatal closure. These facts make ERD15 overexpression plants a very good model for studying light-related phenomena in plant-pathogen interaction. Indeed, our results indicate that the improved resistance of ERD15 overexpressor plants results from increased ROS production. In addition, we take the light-responses of plant pathogens into consideration. Our preliminary results indicate that the growth of various plant pathogenic bacteria is severely compromised by continuous light.

PS13-516

Functional analysis of the interaction between *Puccinia psidii* and Eucalyptus grandis

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Globally, *Puccinia psidii* sl is potentially one of the most dangerous biotrophic fungal pathogens which primarily attacks members of the Myrtaceae, including eucalypts. This pathogen has spread to 4 of the five continents and is currently threatening Australia the largest source of eucalypt germplasm. We designed a model system based on the selection of resistant (R3) and susceptible (S4) plants from a half-sib population using Brasuz1 (Eucalyptus reference genome) as the pollen receptor. First we defined the important steps during the initial interaction between host and pathogen in the resistant and susceptible plants (0-72hr) using microscopy. Based on these data we designed transcript sequencing experiments to cover the most important intervals: 0hr, 6hrs (germination R3 + S4), 12hrs (penetration R3 + S4), 18hrs (resistance process initiates R3), 24hs (resistance process already installed R3 and formation of haustorial mother cells S4) and 72hrs (no pathogen detected R3 and development of secondary hyphae and colonization S4). The transcript sequences were mapped to ESTs from Puccinia species (NCBI) to remove any fungal sequences. The reference data base used for the RNA-seq analysis contained publicly available eucalyptus ESTs and transcripts sequences from the eucalyptus genomic database (Phytozome). RNA-Seq analysis was performed and based on differential expression levels and functional annotation (Blast2GO), 14 candidate genes were selected to validate the sequencing data and propose a model for the interaction between R3 and P. psidii resulting in host resistance.

PS13-517

Regulation of intracellular redox by glyceraldehyde-3-phosphate-dehydrogenase during plant innate immunity

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Glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are important enzymes with diverse cellular regulatory roles in vertebrates, but few reports have investigated GAPDH importance outside of their role in glycolysis in plants. We have found that GAPDHs are upregulated during effector triggered immunity at the protein level. A genetic approach was used to investigate the importance of different GAPDH members during plant innate

immune responses using the interaction between *Arabidopsis thaliana* and the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato*. A subset of GAPDH knockouts exhibit enhanced disease resistance phenotypes. These knockouts show accelerated programmed cell death and increased electrolyte leakage in response to effector triggered immunity. Characterization of reactive oxygen species (ROS) production in some GAPDH knockout lines showed increased ROS production in response to stress elicitors. Additionally, one GAPDH isoform dynamically re-localizes to a site of ROS production during defense responses. These results indicate a role for GAPDHs in cellular redox regulation during plant immune responses against microbial pathogens. ROS are necessary intra-and intercellular signaling molecules and are important for resistance against many types of pathogens. Understanding the mechanisms of ROS flux through regulation by proteins like GAPDHs is important to the elucidation of downstream signaling events during innate immune responses in plants.

PS13-518

Transcriptome analysis of rice leaf and root cells inoculated with *Magnaporthe oryzae*

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Rice plants show a variety of defense responses upon pathogen attack. To understand the responses of rice during the infection by pathogens, we analyzed the gene expression profiles in rice leaves and roots inoculated with *M. oryzae* using rice 44k oligo-DNA array. We used two isogenic rice cv. Nipponbare, NB(*Pia*) and NB(++), which are incompatible and compatible with M. oryzae strain P91-B15, respectively. Hierarchical clustering of microarray results of roots revealed that the most remarkable difference in gene expression profiles between incompatible and compatible interaction was observed at 5 days post inoculation, in contrast to the results where gene expression profiles of leaves in incompatible interaction were most distinct from those in compatible interaction at 3 days post inoculation. It was suggested that these significant differences corresponded to the temporal elongation patterns of infectious hyphae in the leaf and root tissue. Comparison of the results of leaves with the results of roots showed that 155 and 725 genes were differentially regulated in leaf and root cells attacked by *M. oryzae*, respectively. The detailed expression profiles of these genes will be discussed. In addition, we will report the local gene expression changes near infection sites using Laser Microdissection method.

PS13-519

Different responses to FliCs of soft-rot pathogens is attributed to plant species and their sequence composition containing flg22 homologous region

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Our previous data showed that the major flagellar component (FliC) of *Pectobacterium carotovorum* subsp. *carotovorum* EC1 (Pcc-FliC) induced growth inhibition and cell death in tobacco BY-2 cells through the recognition of flg22 homologous region (flg22Pcc) and its downstream residues 51-70 [fliC(51-70)Pcc], respectively. On the other hand, FliC of the other soft-rot pathogen *Dickya dadantii* 3937 (Dd-FliC) did not induce those plant responses. In this study, we examined in responses of *Arabidopsis thaliana* T87 cells and *A. thaliana* seedlings to Pcc-FliC and Dd-FliC. Upon their treatment to *A. thaliana* T87 cells, both FliCs induced cell death and growth inhibition. Furthermore, in the assay using *A. thaliana* seedlings, Dd-FliC induced severer growth inhibition than Pcc-FliC did. In addition, a deletion mutant of Pcc-FliC [Pcc-FliC(δ1-50)], lacking the N-terminal 50 amino acid region containing flg22 homologous region, failed to induce growth inhibition, while the chimeric Pcc-FliC replacing the flg22 homologous region with that of Dd-FliC, [FliC(Pcc1-28/Ddflg22/Pcc51-290)], induced growth inhibition which was indistinguishable from that induced by Pcc-FliC. These results suggested that the recognition mechanism for the FliCs would be different among plant cells or species, and the other regions besides the flg22 homologous region would be involved in induction of the severer growth induction in *A. thaliana* seedlings.

PS13-520

Function of tomato *ERF36* and *ERF39* in response to bacterial wilt and abiotic stress

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Plant constantly encounters abiotic and biotic stresses. For instance, bacterial wilt (BW), which is caused by *Ralstonia solanacearum*, is a very complex deadly disease and shares certain common features with water stress responses. However, information on crop defense mechanisms against this disease is largely not known. Ethylene-response factors (ERFs) are a large family of plant-specific stress responsive transcription factors. Our previous studies revealed that *SlERF3*, a member of group VIII repressor ERFs, plays a positive role in tomato tolerance to *Rs* and salt stress. The aim of this study was to study function of two uncharacterized tomato ERF-VIII members, ERF36 and ERF39, in biotic and abiotic stress responses. Subcellular localization test showed the nuclear localization of GPF recombinant proteins of ERF36 and ERF39 and transactivation assay confirmed the functionality of these proteins as transcriptional repressors. These two genes displayed differential spatial and response expression patterns. Virus-induced gene silencing (VIGS) assays suggested that *SlERF36* and *SlERF39* play a positive role in tomato defense against BW but a negative role in drought tolerance. Overexpression of *SlERF36* or *SlERF39* in transgenic plants led to reduced tolerance to salt and mannitol treatment, further evidencing their negative role in regulation of plant tolerance to water stress. This study reveals function of these genes in both biotic and abiotic stress responses.

PS13-521

Functional study of tomato *ERF35* and *ERF38*

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Plants constantly encounter a wide range of abiotic and biotic stresses, leading to tremendous crop losses. For example, bacterial wilt (BW), which is caused by, *Ralstonia solanacearum*, is a very complex deadly disease and shares certain common features with water stress responses. During evolution, plants have become equipped with versatile defense mechanisms to cope with different stresses and sustain their life. However, our knowledge about how plants coordinate and optimize their machineries to simultaneously maintain physiological functions and reduce stress-caused damages is still rudimentary. Ethylene-response factors (ERFs) are a large family of plant-specific transcription factors involved in various stress responses. The objective of this study was to study function of two uncharacterized tomato ERF-VIII members, ERF35 and ERF38, in biotic and abiotic stress responses. Our data showed that GPF recombinant proteins of ERF36 and ERF39 localize in nucleus and they function as transcriptional repressors. These two genes displayed differential spatial and response expression patterns. Virus-induced gene silencing (VIGS) assays suggested that ,*SlERF35* plays a negative role in defense against BW and drought, and that *SlERF38* plays a positive role in BW defense but a negative role in drought tolerance. To determine whether these genes are also involved in PAMP-triggered immunity (PTI), we have established tomato PTI response assays to treatments

of various PAMPs and *R. solanacearum* related components. Transgenic plants with increased or reduced expression levels of these two genes have been created and being characterized in order to verify their functions in plant stress responses.

PS14-522

Cell-cell sinaling regulates common and contrasting traits in *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonads and *Xylella fastidiosa* shares many genes in common which are involved in the synthesis of a cell-cell communication molecule called as DSF (Diffusible Signal Factor). In *Xanthomonas campestris* pv. *campestris* (Xcc), DSF positively regulates production of type II effectors like endoglucanase, protease as well as EPS. However, DSF appears to regulate iron uptake in *Xanthomonas oryzae* pv. *oryzae* (Xoo) as DSF deficient mutants of Xoo are proficient in EPS and extracellular enzyme production but are deficient in iron uptake. The Xoo DSF deficient mutants overproduce as well as hyper secretes Type II effectors like-cellulase, lipase, xylanase and hyper secretion of these Type II effectors is partly Type II secretion system independent. We hypothesize that over secretion of these cell wall degrading enzymes (which are inducers of plant defense response), may be contributing partly for the virulence deficiency of the *rpfF* mutants of Xoo. The *rpfF* mutants of Xoo are also deficient in forming biofilm, as they are deficient in the synthesis of adhesins and Type I pili. Study of the mechanism of regulation of virulence associated traits by DSF in Xoo indicates that Xoo exhibits atypical mode of regulation by modulating the levels of cyclic Di-GMP (intracellular signal molecule) and novel signal transduction components, which is in contrast to other Xanthomonads to suite its lifestyle.

PS14-523

Tzs is involved in *Agrobacterium* virulence and growth

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Agrobacterium tumefaciens is an organism capable of trans-kingdom DNA transfer, transforming mainly plants but also other eukaryotic species. Genetic transformation by *A. tumefaciens*, which in plants causes neoplastic growths called "crown gall", results from the transfer and integration of a specific DNA fragment (T-DNA) from the bacterium into the plant genome. Here, we characterized a Ti-plasmid encoded gene, *tzs* (*trans*-zeatin synthesizing), that is responsible for the synthesis of a plant hormone cytokinin in *A. tumefaciens* when bacteria were induced by a phenolic compound acetosyringone (AS). To determine the role(s) of *tzs* in *A. tumefaciens* virulence, two *tzs* deletion mutants and three *tzs* frame-shift mutants were generated and characterized. High performance liquid chromatography (HPLC) analyses demonstrated the *tzs* deletion and frame-shift mutants produce no *trans*-zeatin under AS inductions. Both *tzs*-deletion and frame-shift mutants reduce stable and transient transformation efficiency in *Arabidopsis* roots, suggesting that Tzs is likely involved in step(s) prior to T-DNA integrations. The exogenous applications of cytokinin during infections also restored the transient transformation efficiencies in the *tzs* mutants, suggesting that the cytokinin is responsible for the efficient transformation on *Arabidopsis* roots. The *tzs* mutants were able to enhance transformation efficiency on green pepper, and reduce transformation efficiency on white radish and other plant species. These data strongly suggest that Tzs, likely via synthesizing *trans*-zeatin at early stage(s) of infection process, is involved in the transformation efficiency of *A. tumefaciens* and may play different roles in different host plants.

PS14-524

Integrated management of citrus canker disease caused by *Xanthomonas citri* subsp. *citri* in Saudi Arabia

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Citrus canker is one of the most serious diseases affecting citrus production worldwide including Saudi Arabia. Studies were conducted to investigate prospective combinations of indigenous bio-control agents (*Pseudomonas fluorescens* and phage), Serenade, salicylic acid, and Drexide; for the disease management under greenhouse conditions. Combination of salicylic acid (10mM) with *P. fluorescens* and combination of salicylic acid and Serenade with local phage were able to reduce area under disease progressive curve (AUDPC) by 75% and 79% respectively. These combinations did not give a significant difference as compared to Drexide alone, a common control means, which reduced AUDPC by 87.4%. Results suggests that these combination are promising to be used in integrated management of citrus canker disease.

PS14-525

Secretome analysis of rice bacterial blight, *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas oryzae pv. *oryzae* causes bacterial blight (BB) of rice, which is one of the most important diseases of rice in most of the rice growing countries. In plants, bacterial pathogens are unable to penetrate into the host cell. Therefore, molecular signal interaction between microbial pathogen and host occurs in the extracellular space, so called apoplast. In this study, we employed 2-DE analysis to investigate changes in extracellular proteome from BB cultured from *in vitro* and *in vivo*, respectively. Extracellular proteins were isolated by $CaCl_2$ infiltration and applied into 2-D gels. Quantitative and statistical analyses of the resolved spots using ImageMaster software revealed that 153 proteins were differentially up or down regulated from *in vivo* and *in vitro* cultured BB and they were analyzed using MALDI-TOF-MS and μLC-ESI-MS/MS and identified. Among them, 60 spots identified which have signal peptide at their N-terminal region include VirK protein, out membrane protein, TonB dependent receptor, HrcC protein, zinc protease, and several hypothetical proteins. Among the identified proteins, VirK protein, yet unknown function was confirmed by RT-PCR analysis, revealing that the protein up-regulated by *in vivo* cultured BB cells was transcriptionally regulated as well. These results are for the first report, on which *in vivo* specific secreted proteins of BB may be involved in bacterial pathogenecity.

PS14-526

Genomic era of the model soft rot phytopathogen *Pectobacterium* sp. SCC3193 provides surprises in the collection of virulence determinants and the phylogenetic diversity of potato pathogenic soft rot bacteria

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The aim of our work was to enhance understanding of soft rot disease, which is economically one of the most devastating bacterial diseases of plants worldwide. We investigated phylogeny and novelties in virulence in a long time soft rot model strain of *Pectobacterium* isolated from a diseased potato stem in Finland in the early 1980s. We combined genomic approach and in planta experiments to characterize the strain further. We found that the model strain SCC3193 belongs to a different species than was previously thought. We experimentally established novel genes needed for full virulence of *Pectobacterium* on potato. Genome comparison also revealed other interesting traits that may be related to life in planta or needed in other specific environmental conditions. We conclude that the genomic approach and comparison of our soft rot model strain SCC3193 to other sequenced *Pectobacterium* strains, including selected type strains, could provide a solid base for further investigation of the virulence, distribution and phylogeny of soft rot bacteria and maybe other bacteria as well.

PS14-527

Dissociation of bacterial population: a strategy of effective plant colonization

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Poster 14

Many bacterial species colonize plant interior, which consists of various tissues heterogeneous in chemical and immune properties and thus, forms diverse niches for microorganisms. However, the alteration of bacterial population structure in such heterogeneous and changing system is poorly understood. In our research we described the population cycle of plant pathogenic bacterium *Pectobacterium atrosepticum* SCRI1043 (*Pba*) during colonization of *Nicotiana tabacum*. The initial expansion of bacteria inside the plant occurred via xylem vessels. As a result some vessels were completely occluded by bacteria due to formation of specialized multicellular structures, which we called bacterial emboli. Bacterial emboli were composed of cells with specific morphology and had a peculiar way of formation, which included utilization of plant-derived compounds as a component of bacterial extracellular matrix. Colonization of xylem was followed by active propagation of bacteria in parenchyma. Such an order of colonization of various tissues may be related to high immunity of parenchyma cells, which is weakened after vessel occlusion. After plant death, bacteria transformed to viable but non-culturable state. They had ultarstructure typical to dormant cells of *Pba* (Gorshkov et al., 2009), lost the ability to form colonies, but were detected by qPCR-analysis and were able to resuscitate. Part of the population migrated through the root system to rhizosphere employing collective motility. Thus, bacteria realize different programs in different plant tissues and at different stages of plant-microbe interaction. As a whole, this strategy is aimed to effective colonization of plant and successful passing through the population cycle.

PS14-528

Acid-induced ExoR degradation derepresses the ChvG/ChvI two-component system to activate type VI secretion in *Agrobacterium tumefaciens*

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The type VI secretion system (T6SS) is a widespread, versatile protein secretion system in pathogenic Proteobacteria. Several T6SSs are highly regulated by various regulatory systems at multiple levels. However, the signals and/or regulatory mechanisms of many T6SSs remain unexplored. Here, we report on an acid-induced regulatory mechanism activating T6SS in *Agrobacterium tumefaciens*, a plant bacterium causing crown gall disease in a wide range of plants. We monitored the secretion of the T6SS hallmark protein hemolysin-coregulated protein (Hcp) from *A. tumefaciens* and found that acidity is a T6SS-inducible signal. Expression analysis of the T6SS gene cluster comprising the imp and hcp operons revealed barely detected imp expression and Hcp secretion in *A. tumefaciens* grown in neutral minimal medium but highly induced in acidic medium. With loss- and gain-of-function analysis, we demonstrate that the *A. tumefaciens* T6SS is positively regulated by a ChvG/ChvI two-component system and negatively regulated by ExoR. Further epistasis analysis revealed that ExoR functions upstream of the ChvG sensor kinase in regulating T6SS. Interestingly, under acidic conditions, periplasmic ExoR is rapidly degraded, with concomitant increase in ChvG protein level, which is also stabilized with expression of ExoR variants incapable of interacting with ChvG. The phospho-mimic but not wild-type ChvI response regulator can bind to the T6SS promoter region in vitro and activate T6SS with growth in neutral minimal medium. We present the first demonstration of T6SS activation by an ExoR-ChvG/ChvI cascade, with acidity triggering ExoR degradation and thereby derepressing ChvG/ChvI to activate T6SS in *A. tumefaciens*.

PS14-529

Identification of two new type-III effectors in *Xanthomonas oryzae* pv. *oryzicola* required for pathogenesis in rice

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The Gram-negative plant pathogenic bacterium *Xanthomonas oryzae* pv. *oryzicola*, the causal agent of bacterial leaf streak, employs a type-III secretion system (T3SS) to injects repertoires of effector proteins (T3SEs) into rice cells. It has been demonstrated that a consensus sequence motif PIP-box (plant-inducible promoter) recognized by a key *hrp* regulatory protein HrpX was found to present in the promoters of hrp and some of T3SS effector genes. In this study, an T3S effector translocation assay with the biological activity domain of AvrXa10 as a reporter was utilized to evaluate the 29 genes with the PIP box from a gene pool of microarrays. Two novel T3SS effectors, XopAU and XopAV are indentified and verified. XopAU and XopAV were highly conserved in *Xanthomonas* spp. The T3SS secretion of XopAU was not dependent on exit control proteins HpaB and HpaP (HpaC homology), whereas the secretion of XopAV was dependent on HpaB but not HpaP. Moreover, *xopAU* and *xopAV* were induced in a *hrp*-inducing medium XOM3 and regulated by HrpG, HrpX and HrpD6 regulators. The expression of *xopAU* was positively regulated by both HrpG and HrpX whereas negatively regulated by a recently identified hrp regulator HrpD6. In contrast, the expression of *xopAV* was negatively controlled by HrpG, HrpX and HrpD6. Finally, deletion mutagenesis demonstrated that *xopAU* and *xopAV* were required for full virulence of *X. oryzae* pv. *oryzicola* in rice.

PS14-530

A highly-conserved single-stranded DNA-binding protein in *Xanthomonas* Works as a PAMP for PTI

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Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) enables plants to surpass infections by diverse pathogens.

Harpins of Gram-negative plant pathogenic bacteria are considered as one type of PAMPs that typically elicits hypersensitive response (HR) in nonhost plants. However, harpins in *Xanthomonas* species remain largely unknown. We here demonstrated that a highly conserved single-stranded DNA binding protein (SSB) in *Xanthomonas* species, rather than other prokaryotic bacteria, elicits HR in tobacco. SSB is an acid, glycine-rich, cystine-lacking, heat-stable, and proteolysis-sensitive protein. SSB-triggered HR in tobacco is not only a programmed cell death, but also accompanied with active oxygen burst, activation of the expression of HR marker genes and pathogenesis-related protein genes, and callose deposition. SSB-induced HR can be inhibited by eukaryotic metabolism inhibitors, but requires the involvement of BAK1 and BIK1 which are essential for PTI in plants prior to the activation of MAPK and salicylic acid signal pathways. The deletion mutant of *ssb* of *X. oryzae* pv. *oryzicola*, a representative of *Xanthomonas* species causing bacterial leaf streak in rice, reduced bacterial virulence and growth *in planta*, but retained to trigger HR in nonhost tobacco. An imperfect PIP-box (plant-inducible promoter) in the promoter region of *ssb* enables the pathogen to co-express with the key regulator HrpX. In addition, SSB was secreted via the type III secretion system in HrpE-, HpaB- and HpaP-independent manners. This is the first report that the highly-conserved SSB in xanthomonads is positively regulated by HrpX, secreted via T3SS, works as a PAMP for PTI.

PS14-531

Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in Arabidopsis plants

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Phytoplasmas are insect-transmitted bacterial plant pathogens that cause considerable damage to a diverse range of agricultural crops globally. Symptoms induced in infected plants suggest that these phytopathogens may modulate developmental processes within the plant host. We report herein that Aster Yellows phytoplasma strain Witches Broom (AY-WB) readily infects the model plant Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia, inducing symptoms that are characteristic of phytoplasma infection, such as the production of green leaf-like flowers (virescence and phyllody) and increased formation of stems and branches (witches broom). We found that the majority of genes encoding secreted AY-WB proteins (SAPs), which are candidate effector proteins, are expressed in Arabidopsis and the AY-WB insect vector *Macrosteles quadrilineatus*. To identify which of these effector proteins induce symptoms of phyllody and virescence, we individually expressed the effector genes in Arabidopsis. From this screen, we have identified a novel AY-WB effector protein, SAP54, which alters floral development, resulting in the production of leaf-like flowers that are similar to those produced by plants infected with this phytoplasma. This study offers novel insight into the effector profile of an insect-transmitted plant pathogen and reports to our knowledge the first example of a microbial pathogen effector protein that targets flower development in a host.

PS14-532

Hcp2, a secreted protein of the phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000, is required for competitive fitness against bacteria and yeasts

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When analysing the secretome of the plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000, we identified hemolysin co-regulated protein (Hcp) as one of the secreted proteins. Hcp is assumed to be an extracellular component of the type VI secretion system (T6SS). The genome of *Pst* DC3000 harbours two T6SS gene clusters, each of which have one *hcp* gene, named *hcp1* (PSPTO_2539) and *hcp2* (PSPTO_5435). We studied the expression patterns of the *hcp* genes and tested fitness of *hcp* knock-out mutants in host plant colonization and in inter-microbial competition. We found that the *hcp2* gene is expressed and that the expression level is dependent on the bacterial growth phase, reaching the highest level at stationary phase. The Hcp2 protein was found to be secreted into the culture medium, whereas Hcp1 protein was not detected. Expression of *hcp2* was not induced in planta and it did not contribute to virulence or colonization in tomato or *Arabidopsis* plants. Instead, *hcp2* was required for surviving competition with enterobacteria, including *Pectobacterium* species, the soft-rotting plant pathogens, and eukaryotic microbes, such as amoeba and yeast. Deletion of *hcp2* gene abolished the ability of *Pst* DC3000 to inhibit the growth of enterobacteria and yeasts in mixed cultures. For full competitive fitness against yeast *hcp1* was also needed, although playing a minor role compared to *hcp2*. Our results suggest that the T6SS of *P. syringae* may be important for the bacterial fitness in conditions where this plant pathogen has to compete with other micro-organisms for resources.

PS14-533

Virulence determinants of the cucurbit pathogenic bacterium *Acidovorax citrulli*

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The Gram-negative bacterium *Acidovorax citrulli* is the causal agent of seedling blight and bacterial fruit blotch (BFB) of cucurbits. During the last 20 years, serious economic losses caused by BFB have been reported worldwide, primarily in watermelons and melons. Despite the economic importance of the disease, there is little knowledge on basic aspects of *A. citrulli*-host interactions. To identify *A. citrulli* genes associated with pathogenicity, we optimized molecular manipulation techniques and inoculation assays for this bacterium. A transposon mutant library was generated in the background of strain M6 and screened for reduced virulence in seed transmission assays with melon. One of the identified mutants was impaired in production of type IV pili (T4P). Characterization of this and additional T4P mutants revealed that *A. citrulli* requires T4P for twitching motility and wild type levels of biofilm formation and virulence. *A. citrulli* mutants impaired in synthesis of polar flagellum were also shown to possess reduced virulence in various pathogenicity assays. The possible roles of T4P and polar flagella in virulence of this pathogen are discussed. In addition, using marker exchange mutagenesis, we demonstrated that, as similar as other Gram-negative phytopathogenic bacteria, *A. citrulli* requires a functional type III secretion system to secrete virulence effectors into host cells and promote disease. We are currently investigating the role of various type III-secreted (T3S) effectors in the virulence of *A. citrulli*. Here we also report on the genetic variability of T3S effector genes from a collection of strains isolated from different hosts and locations.

PS14-534

Global genes expression profiling of *Xanthomonas axonopodis* pv. *glycines* 12-2 during infection in soybean

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Xanthomonas axonopodis pv. *glycines* strain 12-2 (Xag) causes pustule disease on soybean. To identify the genes in Xag that are altered in expression during infection of soybean plants compared to growth in a minimal medium *in vitro*, Xag draft genome was developed and transcriptome analysis using deep RNA sequencing of mRNA was performed. Of 5062 predicted genes in the Xag draft genome, 534 genes were identified as being up-regulated in the plant while 289 were down-regulated. Plant up-regulated genes included the *hrp* cluster, and genes encoding avirulence and type III effector proteins, extracellular enzymes, chemotaxis components, detoxification, nutrient acquisition and several other known or new putative virulence factors. Plant down-regulated genes included those involved in attachment and the movement process. This study is the first to report on Xag genes expression during infection of soybean and the insights into the behavior of the pathogen should prove useful for developing strategies for controlling disease in this important agricultural crop.

PS14-535

Transcriptional control of Arabidopsis responses to the pathogenic effector protein AvrRpm1 from *Pseudomonas syringae*

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Bacterial- and fungal pathogens secrete effector proteins into host plant cells to increase virulence. Plants, on the other hand, have resistance proteins whose function is to counteract the activity of pathogenic effectors. Wild type *Arabidopsis thaliana* recognize the *Pseudomans syringae* effector AvrRpm1 and induces the so called hypersensitive response (HR). The mutant rpm1 does not recognize the effector and in this background the effector enhance the virulence of *P. syringae*. In this study we investigated the expression changes of transcription factor genes in Arabidopsis as a response to the AvrRpm1 in the presence or absence of its cognate resistance gene RPM1. In contrast to previous studies, we detected transcriptional responses already 15 min after the induction of expression of a bacterial effector protein *in planta*. As a result, we provide a detailed view of the early changes of transcription factor gene expression in response to the AvrRpm1 action *in planta*. The data shows that plant resistance responses and pathogenic driven disease responses each have unique transcriptional responses. We have thus, expanded the list of transcriptional regulators potentially involved in controlling plant resistance responses and identified possible direct and/or indirect targets of the virulence function of AvrRpm1. Finally, a subset of the identified transcription factor genes was selected for a reverse genetics approach. No single knock out mutant displayed severe resistance phenotypes. However, a few knock out mutants did demonstrate changes in cell death or expression of pathogenesis related protein 1 after inoculation with avirulent *P. syringae*.

PS14-536

Specific induction mechanism of rice immune responses by flagellins from *Acidovorax avenae*

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Acidovorax avenae is a gram-negative plant pathogenic bacterium. The flagellin from *A. avenae* rice avirulent N1141 strain induced several plant immune responses including H_2O_2 generation, while the flagellin from rice virulent K1 strain did not. To clarify molecular mechanism that leads to these differing between the N1141 and K1 flagellins, recombinant N1141 and K1 flagellins were generated using an *Escherichia coli* expression system. When cultured rice cells were treated with recombinant K1 or N1141 flagellin, both flagellins equally induced H_2O_2 generation, suggesting that post-translational modifications of the flagellins are involved in the specific induction of immune responses. Mass spectral analysis and glycan analysis showed that 1,600 and 2,150 glycans were present on the N1141 and K1 flagellins, respectively. A deglycosylated K1 flagellin induced H_2O_2 generation in the same manner as N1141 flagellin. Tryptic peptide mappings with reverse-phase HPLC and site-directed mutagenesis revealed that glycans were attached to four amino acid residues (^{178}Ser, ^{183}Ser, ^{212}Ser and ^{351}Thr) in K1 flagellin and three amino acid residues (^{178}Thr, ^{183}Thr and ^{351}Thr) in N1141 flagellin. Among mutant K1 flagellins in which each glycan-attached amino acid residue was changed to alanine, ^{178}Ser/Ala and ^{183}Ser/Ala K1 flagellins induced a strong immune response in cultured rice cells, indicating that the glycans at ^{178}Ser and ^{183}Ser in K1 flagellin prevent epitope recognition in rice.

PS14-537

The role of two-component response regulator in biofilm formation and pathogenicity by *Xanthomonas axonopodis* pv. *citri*

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Citrus bacterial canker caused by *Xanthomonas axonopodis* pv. *citri* is a serious disease impacting on citrus production worldwide. Biofilm formation has been indicated to be important for various bacteria to successfully develop pathogenic relationships with their host. To understand the mechanisms of biofilm formation by *X. axonopodis* pv. *citri* strain XW19, the strain was subjected to transposon mutagenesis. One mutant with mutation in two-component regulatory protein, TCR, and deficient in biofilm formation in polystyrene microplate was selected for further study. The strain XW19 TCR shares 100% amino acid sequence identity with XAC1284 of *X. axonopodis* pv. *citri* strain 306 and 84-100% identity with two component regulatory proteins in various pathogens and environmental microorganisms. Biofilm formation by TCR mutant was significantly decreased on leaf surfaces of Mexican lime compared to that by the wild type. TCR mutant was compromised in its ability to cause canker lesions. The wild-type phenotype was restored by providing pTCRF1, pTCRF2 *in trans* in TCR mutant. Our data indicated that TCR did not regulate the production of virulence-related extracellular enzymes including amylase, lipase and lecithinase, or expression of *hrpG*, *rfbC*, and *katE*, while controlled the expression of *rpfF* in XVM2 medium which mimics cytoplasmic fluids *in planta*. In conclusion, biofilm formation on leaf surfaces of citrus is important for canker development by *X. axonopodis* pv. *citri* XW19. The process is controlled by two-component regulatory protein TCR through regulation of *rpfF*, which is required for biosynthesis of diffusible signal factor.

PS14-538

Involvement of a phosphinothricin N-acetyltransferase gene in virulence diversity of *Pseudomonas cichirii* strain SPC9018

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Pseudomonas cichirii strain SPC9018 (SPC9018) harbors the *hrp*. The *hrp* mutants from SPC9018 lose their virulence on eggplant but not lettuce. *P. cichorii* acquires the *hrp* through the horizontal gene transfer from a common ancestor with the single pathogenicity island (S-PAI) in *P. viridiflava*. A phosphinothricin N-acetyltransferase gene (*pat*) is located in the flanking regions of the *hrp* in both *P. cichorii* and the S-PAI. Phylogenetic analyses suggested that *P. cichorii* might acquire *pat* through the horizontal gene transfer from the donor common to the S-PAI. The *pat*-deficient mutant from SPC9018 lost its virulence on eggplant but not lettuce. The mutant grew slower in eggplant leaves, compared to SPC9018. Expression of *pat* in SPC9018 was not regulated by HrpL, the transcriptional activator for the *hrp*. On the other hand, deletion of *pat* resulted in a decrease in the *hrp* expression, which depended on the bacterial density. These results suggest that *pat* is involved in growth of the bacteria in planta and the *hrp* expression, leading to implication of *pat* in the bacteria virulence on eggplant. Inoculation into *P. cichorii*-susceptible Asteraceae species including lettuce showed that the involvement of the *hrp* and *pat* in *P. cichorii* virulence is independent of each other and has no relationship with the phylogeny of Asteraceae species based on the nucleotide sequences of *ndhF* and *rbcL*. Taken together, after acquisition of the *hrp* and *pat* of which a PAI consists, *P. cichorii* implicates the *hrp* and *pat* in its virulence diversity on respective *Asteraceae* species.

PS14-539

Involvement of a lectin gene, *fml*, in virulence of *Ralstonia solanacearum* strain OE1-1

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The *hrp* genes (*hrp*) is required for pathogenisity of *Ralstonia solanacearum* strain OE1-1 (OE1-1). Through a complex multigene regulatory cascade PrhA-PrhR/PrhI-PrhJ-HrpG, *hrp*B expression is induced in response to contact with plant cells. The transcriptional regulator HrpB activates the entire *hrp*. PhcA is a LysR-type transcriptional regulator activated by a quorum-sensing system. At high cell density, activated PhcA not only induces synthesis of extracellular polysaccharide, which is the determinant of the bacterial virulence, but also inhibits *hrp* expression through *prhIR* repression. In this study, we first performed the transcriptome analysis with the next generation sequencer using the *phcA*-mutant, suggesting that a lectin gene, *fml*, is positively regulated by PhcA. Valls *et al*. (2006) have demonstrated that *fml* expression is positively regulated by HrpG. The RT-PCR analysis confirmed that *fml* expression depends on not only HrpG at low cell density but also PhcA at high cell density. The *fml* mutation resulted in low growth ability of the bacteria in tomato plants and reduced virulence of the bacteria on tomato. Therefore, *fml* is included in both the *hrp* regulon and the PhcA regulon, and is implicated in *in planta* growth of OE1-1 and its virulence. Furthermore, the *fml*-mutant showed higher *fliC* expression, resulting in higher swimming motility, compared to OE1-1. The *fml* mutation resulted in reduced biofilm productivity in the poor medium, and enhanced biofilm productivity in the rich medium. Results in this study also suggest involvement of *fml* in swimming motility and biofilm productivity.

PS14-540

Functional roles of VirB2 in the type IV secretion system, T-pilus, and virulence of *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens is a phytopathogenic bacterium which causes crown gall disease by transferring T-DNA into the host genome. The translocation process is mediated by the type IV secretion system (T4SS) comprising the VirD4 coupling protein and 11 VirB proteins (from VirB1 to VirB11). All VirB proteins are required to assemble the T-pilus, which consists of processed VirB2 (T-pilin) as a major subunit. While VirB2 is an essential component of T4SS, the roles of VirB2 and the assembled T-pilus remain unknown. Here, we generated a series of VirB2 amino acid substitution mutants to study the mechanistic functions of VirB2 involved in the assembly of T4SS, T-pilus and virulence of *A. tumefaciens*. Based on the ability in T-pilus production and tumorigenesis on tomato stems, three major classes of mutants (T-pilus$^-$/Vir$^-$, T-pilus$^-$/Vir$^+$, and T-pilus$^+$/Vir$^+$) were isolated. All mutations in the first trans-membrane domain of processed VirB2 resulted in severe defects on T-pilus production, suggesting the importance of this domain for T-pilus biogenesis. We also identified several T-pilus$^-$/Vir$^+$ uncoupling mutants, consistent with previous conclusion that T-pilus does not play an essential role for virulence. However, while these uncoupling mutants remain wild-type level of tumorigenesis efficiency on potato tuber discs, they are highly attenuated in transient transformation efficiency in *Arabidopsis* seedlings. In conclusions, we provided the first demonstration for a role of T-pilus in T-DNA transformation process and revealed the domains and amino acid residues critical for T4SS/T-pilus assembly and virulence of *A. tumefaciens*.

PS14-541

Hemin transported protein of *Xanthomonas axonopodis* pv. *glycines* functions on leaf colonization and virulence on soybean

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Xanthomonas axonopodis pv. *glycines* (Xag) causes bacterial pustule disease on soybean. This bacterium is worldwild spread around hot and humid growing region likes in Southeast Asia. To understand the gene coding for hemin transporter protein (*hem*) involved in virulence of the pathogen in soybean, we generated a *hem* mutant in Xag by overlapping PCR mutagenesis. Disruption of *hem* significantly reduced the disease incidence when sprayed on soybean but not function when it was injected directly into plant. The *hem* mutant caused the hypersensitive response induction on tobacco as Xag wildtype. Interestingly, the *hem* expression was also reduced when Xag wildtype grew *in planta*. The hemin transporter protein involved in the production of extracellular polysaccharide, biofilm formation, motility and attachment but not for extracellular enzymes. This confirmed that epiphytic fitness by Xag strongly required hem function. The result suggests that *hem* gene is essential for virulence of Xag on soybean during infection process.

PS14-542

Biosynthesis of diffusible signal factor (DSF) signals in *Xanthomonas campestris* pv. *campestris* is induced by host metabolites

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Quorum sensing (QS) denotes a widely conserved cell-to-cell communication mechanism which coordinates bacterial group behavior and often regulates virulence, biofilm formation, antibiotic production and plasmid conjugal transfer. The cell-cell communication signal cis-11-methyl-2-dodecenoic acid (also known as DSF) was originally identified in *Xanthomonas campestris* pv. *campestris* (Xcc), representing a widely conserved signaling mechanism in many Gram-negative bacterial pathogens. The signal is involved in the regulation of biofilm dispersal and virulence. Previous work showed that DSF biosynthesis in Xcc is dependent on RpfF and RpfB, but it is not clear how host may affect its production. Here we report that exogenous addition of the cell-free extract from Chinese cabbage to the growth medium of Xcc significantly induces the DSF-family signal production. The further study showed that the biosynthesis of BDSF and DSF are significantly enhanced. Our works reveal that Xcc can utilize host metabolites in Chinese cabbage to increase quorum sensing signal production, and facilitate its infection.

PS14-543

Functional characterization of genes encoding HD-GYP domain proteins in *Xanthomonas oryzae* pv *oryzicola*

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Bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) is one of important diseases in rice. However, little is known about the pathogenicity mechanisms of *Xoc*. Here, we investigated the function of the three Xoc HD-GYP proteins including RpfG in biofilm formation, the production of extracellular polysaccharides, the secretion of extracellular enzymes, as well as virulence on rice. Deletion of *rpfG* resulted in decreased production of extracellular polysaccharides, abolished *Xoc* virulence on rice, but enhanced biofilm formation. Biochemical studies including colorimetric assays, HPLC and mass spectrometry demonstrated that RpfG is a phosphodiesterase that hydrolyses c-di-GMP into GMP via linear pGpG as an intermediate degradation product. Cross-complementation of the *Xoc rpfG* mutant with *rpfG* from *X. campestris* (*Xcc*) restored the mutant phenotypes, but *Xoc rpfG* did not cross-complement the *Xcc rpfG* mutant in EPS production and the secretion of extracellular enzymes. Expression analysis showed that deletion of *rpfG* significantly increased expression of the type III secretion system (T3SS) and *pgaABCD* operon that is required for biofilm formation in *Escherichia coli*. The other two HD-GYP domain proteins have no effect on virulence factor synthesis and tested phenotypes. The results indicated that RpfG in *Xoc* positively controls the production of extracellular polysaccharides and virulence on rice, but negatively regulates biofilm formation and T3SS expression. The results also suggested that the *pgaABCD* operon is likely involved in biofilm production in *Xoc*.

PS14-544

Interactions of HrpB proteins in *Xanthomonas oryzae* pathovar *oryzae*

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Xanthomonas oryzae pathovar *oryzae* is the causal agent of rice bacterial blight disease. The type III secretion system of *X. oryzae* pathovar *oryzae*, encoded by *hrp* (hypersensitive response and pathogenicity) gene cluster, is necessary for both pathogenicity in susceptible hosts and the induction of the hypersensitive response in resistant. In this cluster, we were focusing at the function of HrpB proteins encoded by the *hrpB* operon - HrpB1, HrpB2, HrcJ, HrpB4, HrpB5, HrcN, HrpB7 and HrcT, which were not well characterized except HrcN as ATPase. We hypothesized that these HrpB proteins may work together, so we tested the interactions among these eight proteins by yeast two-hybrid. We cloned these eight *hrpB* genes to bait vector-pGBKT7 and prey vector-pGADT7. We carried out co-transformation with these eight baits and eight preys to yeast strain AH109 and 64 combinatorial tranformants were formed. Sixty-four yeast transformants were tested about livability on the auxotroph medium and blue color on the x-α-gal plate. As a result, HrpB2, HrpB4, HrpB5, HrcN and HrpB7 proteins have interactions among them, but, HrpB1, HrcJ and HrcT proteins have no interactions. Specially, in case HrpB2, HrpB5, HrcN and HrpB7 proteins showed self-interactions. So we suggest that the proteins encoded by *hrpB* operon work together to their plant pathogenic function.

PS14-545

Substrate specificity switching during type III secretion in the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria*

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The Gram-negative bacterial plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) utilizes a type III secretion (T3S) system to translocate a large set of bacterial effector proteins directly into the eukaryotic cell. The T3S system is a highly complex nanomachine that spans both bacterial membranes and is associated with an extracellular pilus and a predicted translocon in the plant plasma membrane. Given the architecture of the T3S system, it is assumed that pilus and translocon formation precedes effector protein translocation and that there is a switch in the T3S substrate specificity from early to late substrates. T3S substrate specificity switching in *Xcv* presumably depends on the switch protein HpaC and the cytoplasmic domain of the inner membrane protein HrcU ($HrcU_C$), which is autoproteolytically cleaved and is presumably involved in the recognition of secreted proteins. $HrcU_C$ interacts with HpaC and the early T3S substrate HrpB2, which is required for pilus assembly and probably functions as a periplasmic component of the T3S system at the base of the pilus. A predicted conformational change in $HrcU_C$ that presumably occurs after the binding of HpaC leads to the substrate specificity switch after pilus formation. Interestingly, the results of mutant and interaction studies suggest that HrpB2 and HpaC compete for the same binding site in HrcU and that the secretion of early and late substrates is controlled by different mechanisms that can be uncoupled.

PS14-546

Variations in type III effector repertoires do not correlate with differences in pathological phenotypes and host range observed for *Xanthomonas citri* pv. *citri* pathotypes

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Xanthomonas citri pv. *citri* (Xac) is a quarantine bacterium causing Asiatic citrus canker. Strains of Xac are classified as pathogenic variants i.e. pathotypes, according to their host range: strains of pathotype A infect a wide range of rutaceous species, whereas strains of pathotype A*/A^w infect a restricted host range consisting of Mexican lime (*C. aurantifolia*) and alemow (*C. macrophylla*). Based on a collection of 55 strains we investigated the role of type III effectors (T3E) in host specialization. By PCR we screened 56 *Xanthomonas* T3Es and showed that Xac possesses a repertoire of 28 effectors, 24 of which are shared by all strains, while 4 (*xopAI, xopAD, xopAG* and *xopC1*) are present only in some A*/A^w strains. However, their distribution could not account for host specialization. *XopAG* is present in all A^w strains, but also in three A* strains genetically distant from A^w , and all *xopAG*-containing strains induced HR-like reactions on grapefruit and sweet orange. A strains are genetically less diverse, induce identical phenotypic responses, and share exactly the same T3Es. Conversely, A*/A^w strains exhibited a wider genetic diversity in which clades correlated to geographical origin and T3Es repertoire but not to pathogenicity. A*/A^w strains showed a broad range of reactions on several *Citrus*, but genetically related strains did not share phenotypic responses. Our results showed that A*/A^w strains are more variable (genetically and pathogenetically) than initially expected and that this variability should not be ignored when trying to describe mechanisms involved in the pathogen evolution and host specialization.

PS14-547

Regulons of *expA* and *rsmA* in *Pectobacterium* strain SCC3193

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Two regulatory genes; *rsmA* and *expA* are well distributed among many enterobacteria and have been extensively studied to date. These two genes have been shown to have an impact on functions such as metabolism, motility and virulence. They have also been linked to the same regulatory pathway when concerning the production of plant cell-wall degrading enzymes (PCWDEs), with *expA* controlling *rsmA* expression through genes such as *rsmB*. The genome of the *Pectobacterium* strain SCC3193, was recently sequenced. Previous work with this strain has revealed that knock-out mutants in *expA* exhibit highly reduced virulence on plants, along with reduced production of PCWDEs. In contrast, knock-out mutants of *rsmA* in SCC3193 demonstrate increased production of PCWDEs, and increased expression of many virulence related genes. Since previous studies indicate that *expA* and *rsmA* operate in the same pathway of regulating the production of PCWDEs, we wanted to further explore the overlap of their regulons and to determine if *expA* exerts its influence through *rsmA* in other aspects of bacterial physiology. We have thus conducted gene expression microarray experiments to determine the transcriptome of knock-out mutants in *expA* and *rsmA*, as well as a double knock-out mutant. Our work reveals synergies and divergence of the transcriptomes of these two genes involved in global genetic regulation, with impact on genes directly and indirectly involved in virulence. In addition to the microarray we have performed assays of growth, virulence and enzyme production, linking the transcriptomic differences of the mutants to phenotypic differences in virulence.

PS14-548

Quorum sensing mechanism mediates virulence control in the plant pathogen *Xylella fastidiosa*

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The xylem-limited plant pathogen *Xylella fastidiosa*, that colonizes the grape vascular system employs a Diffusible Signal Factor (DSF) to control virulence. DSF is synthesized by RpfF and sensed by the RpfCG phosphorelay system that modulates cyclic di-GMP metabolism that serves as a switch to transition between a motile plant-colonizing phase and a more adhesive, non-motile form that can be vectored by insect vectors. *rpfF* and *rpfG* mutants migrate faster in the plant, proliferate more, cause more symptoms, are less "sticky" than the wild type strain, but are not transmissible; both mutants exhibit lower expression of traits contributing to biofilm formation such as hemagglutinin-like proteins including HxfA and higher expression of genes associated with motility, growth and proliferation. DSF consists of one or more unsaturated fatty acids including 2-Z-tetradecanoic acid DSF; it is active at concentrations as low as 1 μM as measured using *hxfA::phoA* transcriptional fusions in *X. fastidiosa*. In addition, adhesiveness of *X. fastidiosa* increased while growth was suppressed in response to exogenous synthetic 2-Z-tetradecanoic acid. We propose that DSF anti-virulence activity may have evolved to avoid excessive colonization of xylem vessels that is lethal to *X. fastidiosa*. Disease control can be achieved in a process of pathogen confusion in which DSF levels are elevated in plants in advance of pathogen infection by topical application and by expression of *rpfF* in transgenic grape.

PS14-549

Identifying factors involved in pathogenicity of *Ralstonia solanacearum* strains at low temperatures using a proteomics approach

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Ralstonia solanacearum species is well adapted to life in subtropical and tropical regions thus most of the populations are non-pathogenic below 20^{o}C, however R3B2 strains not established in the U.S. have been identified to cause disease at low temperatures. Due to risks associated with cool virulent strains, knowledge regarding pathogenicity at low temperatures is needed to facilitate effective disease control. In order to identify putative proteins/pathways possibly involved in pathogenicity at low temperatures, we compared protein levels of two strains of *R. solanacearum* that are not naturally pathogenic at low temperatures (P597, GMI1000) and two strains that are cool virulent (P673, UW551) at 30^{o}C and 18^{o}C. Proteins were extracted and 2-D DIGE protein gels were run in several experiments. Comparisons were made for cellular and secreted proteins when *R. solanacearum* cells were incubated in co-culture with tomato seedlings grown *in vitro* in liquid medium, focusing our attention to the root colonization phase of disease progress. The differential profiles of various comparisons in 5 experiments produced 164 proteins mostly involved with survival in a hostile environment. After exhaustive analysis 29 unique proteins were identified as best potential candidates for cold virulence factors. The candidates include a catalase, PilQ, exoglucanase A, a drug efflux pump, and two hypothetical proteins. Currently we are confirming differential regulation of selected candidates using qRT-PCR and testing their putative role in virulence at low temperature. Preliminary experiments suggest that virulence at 30^{o}C of P673 PilQ defective mutants is not significantly reduced; however it is at 18^{o}C.

PS14-550

Experimental evolution of host specificity in *Pseudomonas syringae*

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The TTSS is required for virulence in pathogenic *P. syringae*, and the structural components of the secretion system are highly conserved across all pathovars, though there is considerable variation in the effector complement of each strain. While the suite of effectors delivered into the host cell cytosol clearly plays an important role in determining its virulence, additional factors such as toxins, adhesins and host innate immunity elicitors also contribute to *P. syringae* virulence and host range. I employed an experimental evolution approach to identify the genetic bases of host specificity in *P. syringae* and elucidate whether there are multiple evolutionary trajectories to pathogenicity on a single host. Replicate isogenic populations of Pph1448A were serially passaged in the Moneymaker cultivar of tomato for 12 weeks. Although Pph1448A has a functional TTSS and can successfully infect bean, it is nonpathogenic on tomato. Not only do multiple evolved populations exhibit higher growth than the ancestral Pph1448A, some grow nearly as well as the tomato pathogen PtoDC3000. Interestingly, most evolved populations maintain maximal bacterial densities between day 3 and 7, rather than experiencing population decline characteristic of PtoDC3000. In addition, no Pph1448A derived lines produce the phenotypic symptoms of necrosis and chlorosis characteristic of PtoDC3000 infection, indicating heightened levels of host damage do not accompany their enhanced growth in tomato. Illumina paired-end sequencing of evolved population revealed the presence of multiple mutations, including a SNP in the transmembrane domain of FlhA, a component of the flagellar type III protein export apparatus.

PS14-551

N-acetyl-L-cysteine prevents *Xylella fastidiosa* colonization in citrus plant, thereby decreasing virulence

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Citrus production is one of the main Brazilian agribusiness activities. However, citrus production in Brazil is greatly affected by diseases. The Citrus Variegated Chlorosis (CVC) caused by the bacterium *Xylella fastidiosa* (*Xf*) is one of the most important citrus diseases. *Xf* multiply and attach to the xylem vessels, forming biofilm that can block water and nutrient transport. Disulfide bonds play an important role in folding and stability of fimbrial and afimbrial proteins, which are important factors for colonization and biofilm formation. N-acetyl-L-cysteine(NAC), an analogue of cysteine can disrupt these bonds being used in treatment of bacterial human diseases. We previously demonstrated that NAC can disrupt *Xf* biofilm *in vitro*, decrease EPS production and significantly decrease the symptoms of CVC *in planta* (hydroponic system). Thus to verify the potential use of this molecule in field condition we evaluated the effects of NAC applied by fertigation system on the (i) evolution of CVC symptoms, (ii) movement and colonization of *Xf*,and (iii) population of *Xf*, comparing sweet orange plants treated or not with NAC. After six months with weekly NAC treatment there was a significant symptoms reduction in plants treated with NAC, mainly in leaves showing chlorosis but not necrosis. A significant reduction in the bacteria population was observed for plants treated with NAC. These results indicate that NAC may have an effect on *Xf* population and the symptoms remission could be a possible consequence of restoration of the xylem flow, opening a new perspective for its use on *Xf* control.

PS14-552

Depriving sugars from apoplast located bacterial pathogens by regulating nutrient efflux is a plant defense strategy and is a component of nonhost resistance

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Nonhost resistance (NHR) is the resistance of a plant species against a wide range of pathovars of a particular pathogen. Unlike R gene-mediated resistance, NHR mechanism is not well studied. Unraveling NHR mechanism may help us develop plants with durable disease resistance against a broad spectrum of pathogens. We have identified a role for sucrose transporter, NbSUT1, in NHR from a virus-induced gene silencing-based forward genetic screen in *Nicotiana benthamiana*. Plants silenced for NbSUT1 compromised NHR against *Pseudomonas syringae* pv. *tomato* T1. The apoplast of the silenced plants had higher levels of various sugars compared to non-silenced control plants. Apoplastic fluid from the silenced plants supported higher *in vitro* growth of several bacterial pathogens. Gene expression profiling of AtSUC2 gene, an Arabidopsis homolog of NbSUT1 gene, and other AtSUC family members in Arabidopsis treated with various host/nonhost pathogens and pathogen associated molecular patterns (PAMPs) indicated that expression of some of the sucrose transporter family members were induced. We hypothesize that few AtSUC gene family members can be targeted by a host pathogen to favor its growth in the apoplast. Using interaction studies of several *Pseudomonas* strains with two model plants, *N. benthamiana* and Arabidopsis, we explain the broader concept for the regulation of sucrose efflux into phloem in the source leaf tissue during pathogen infection. Based on this study and one of our previous studies (Plant Physiology 158; 1789-1802), we will present a model explaining the importance of nutrient regulation as a component of NHR in plants.

PS14-553

Type IV pilin is glycosylated in *Pseudomonas syringae* pv. *tabaci* 6605 and required for surface motility and virulence

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Type IV pilin (PilA) is a major constituent of pilus and is required for bacterial biofilm formation, surface motility, and virulence. It is known that mature PilA is produced by cleavage of the short leader sequence of the pilin precursor, followed by methylation of N-terminal phenylalanine. The molecular mass of the PilA mature protein from the tobacco bacterial pathogen *Pseudomonas syringae* pv. *tabaci* 6605 (*Pta* 6605) was predicted to be 12,329 Da from its deduced amino acid sequence. We previously detected PilA pilin as an approximately 13 kDa protein by immunoblot analysis with anti-PilA-specific antibody. In addition, we found the putative oligosaccharide-transferase gene *tfpO* downstream of *pilA*. These findings suggest that the PilA in *Pta* 6605 is glycosylated. The defective mutant of *tfpO* showed reductions in pilin molecular mass, surface motility, and virulence toward host tobacco plants. Thus pilin glycan plays important roles in bacterial motility and virulence. On the other hand, the genetic region around *pilA* was compared among *P. syringae*. The *tfpO* gene exists in some strains of pvs. *tabaci, syringae, lachrymans, mori, actinidiae, maculicola*, and *P. savastanoi* pv. *savastanoi*. However, some strains of pvs. *tabaci, syringae, glycinea, tomato, aesculi*, and *oryzae* do not possess *tfpO*, and the existence of *tfpO* is independent of the classification of pathovars/strains in *P. syringae*. Interestingly,

the PilA amino acid sequences in *tfpO*-possessing strains showed higher homology with each other than with *tfpO*-non-possessing strains. These results suggest that *tfpO* and *pilA* might co-evolve in some specific bacterial strains.

PS14-554

A novel non-ribosomal peptide synthetase is required for pathogenicity of *Pectobacterium* on potatoes

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Pectobacterium carotovorum subspecies *brasiliensis* (*Pbr*) is an aggressive phytopathogen of potato causing blackleg of stems and soft rot of tubers. Originally detected in Brasil, *Pbr* was recently identified in South Africa and New Zealand. Genome sequencing of *Pbr* NZEC1, a highly virulent isolate collected from potato in New Zealand, revealed several putative virulence factors encoded on large segments of DNA predicted to have been acquired by horizontal gene transfer. These regions, known as Horizontally Acquired Islands (HAIs), are inserted into the bacterial chromosome. Comparative genomics of *Pbr* NZEC1 with other *Pectobacterium* strains showed that one HAI carries a novel non-ribosomal peptide synthetase (NRPS) cluster, which is present in all blackleg-causing strains of *Pectobacterium* (e.g. *Pectobacterium atrosepticum* SCRI1043) but absent in *Pectobacterium* strains unable to cause blackleg. To assess the role of the NRPS in virulence of both *Pbr* and the related blackleg-causing species *P. atrosepticum*, genes encoding the synthetase and its related ABC transporter were inactivated. Pathogenicity assays were subsequently carried out on potato stems and tubers using the resulting knockout mutants. Inactivation of genes in the NRPS cluster abolished the ability of both *Pectobacterium* species to cause blackleg as well as soft rot, demonstrating the importance of the NRPS in virulence of these pathogens. Since, the NRPS shows homology to syringomycin, a putative phytotoxin involved in causing necrosis in host plants by other plant pathogens, we predict that the peptide synthetase is an important virulence factor in all blackleg-causing *Pectobacterium* species.

PS14-555

Identification and characterization of *Streptomyces* spp. causing potato common scab in Vietnam

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The identification and characterization of *Streptomyces* spp. causing common scab of potato in northern Vietnam was for the first time investigated. Fifty eight *Streptomyces* spp. isolates were collected from disease lesions of potato tubers harvested from 4 different provinces of Vietnam. Base on morphological, arial mycellium and diffusible melanoid pigment ability on ISP mediums, they were classified into 8 groups. These groups were characterized by white to grey colonies with spiral or flexuos spores chain. From these 8 groups, 12 representative isolates were amplified with species-specific PCR primers. Among that, 7 isolates were identified as *S. bottropensis*, 3 isolates as *S. Stelliscabies*, 1 isolate as *S.turgidiscabies*, 2 isolates as *S.* group X and 2 isolates have PCR product with *S. scabies*/ *S. euroscabies* specific primers. Cloning and sequencing the 16s rRNA showed they were closely related to known pathogenic *S. scabies* strains and there were sequence variation in three regions. Pathogenicity- and virulence-related genes (*txtAB*, *nec1*, *tomA*) were PCR-amplified from each isolate showing that all of them lack the *nec1* gene, 1 isolate has *txtAB* and 4 isolates have *tomA*. Using these isolates for pathogencity testing showed that they all induced common scab lesion on variety Atlantic with different degrees of severity even with the isolates that lack all main genes of PAI. We proposed that there may have different pathogen strains inducing common scab on potato in Vietnam. The results of this study contribute the additional complexity in the pathogenic strains causing potato common scab disease in the world.

PS14-556

Iron acquisition by phosphinothricin *N*-acetyltransferase-regulated siderophore may be one of determinants for virulence of *Pseudomonas cichorii*

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Pseudomonas cichorii strain SPC9018 (SPC9018) harbors *hrp* which is involved in virulence diversity of the bacteria. A phosphinothricin *N*-acetyltransferase gene (*pat*) is located in the flanking region of *hrp* in the genome of *P. cichorii*. Our previous study showed that *pat* is implicated in virulence diversity of the bacteria, independent of *hrp*. It is thought that a pathogenicity island in *P. cichorii* consists of *hrp* and *pat*, which are acquired through horizontal transfer from the donor common to the single pathogenicity island of *P. viridiflava*. To elucidate function of *pat* on virulence of *P. cichorii*, phenotypical characteristics of the *pat*-deleted mutant of SPC9018 were analyzed. Swarming motility of the *pat*-deleted mutant in media with $FeCl_3$ reduced, compared to that without $FeCl_3$, suggesting involvement of *pat* in iron acquisition. Therefore, we then analyzed the siderophore activity by the *pat*-deleted mutant. At low cell density, the *pat*-deleted mutant showed weaker siderophore activity, compared to the SPC9018. The deletion of *pat* resulted in reduced expression of siderophore pyoverdine synthesis-related *pvdL* and production of the pyoverdine. Interestingly, co-treatment of 20 μM mugineic acid, an iron chelator, with SPC9018 resulted in suppression of *in planta* growth of the bacteria. Furthermore, co-treatment of mugineic acid resulted in loss or reduction of SPC9018 virulence on *P. cichorii*-susceptible plants including eggplant, on which *pat* is involved in virulence of the bacteria. Results in this study suggest that iron acquisition by *pat*-regulated siderophore productivity may be one of determinants for virulence of *P. cichorii*.

PS14-557

Identification of novel effectors in *Pseudomonas syringae* pv. *actinidiae* the causal agent of kiwifruit canker

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An incursion of the devastating disease of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Pan) was detected in New Zealand in November 2010. This pathogen is exceptionally virulent and has caused significant damage to the kiwifruit industry in Europe over the past three years. The infection rapidly becomes systemic resulting vine death within a matter of weeks. There are currently no methods for controlling the disease. A whole genome sequencing project, with the aim of analysing the sequences of over 40 isolates of Pan from around New Zealand and overseas has been completed. Phylogenetic analysis of these sequences based on 400 genes conserved in *Pseudomonas*, indicates that the virulent

isolate (Pan-V) is most closely related to the Pan isolates first found in Japan. Although very closely related phylogenetically, the Pan isolates from Japan and New Zealand are significantly different in their complement of effectors and pathogenicity factors. We have compared the genome arrangement of these related isolates using a combination of Illumina and 454 sequencing. An analysis of the effector complement of Pan-V indicates the presence of two effectors, co-located in the genome, and a putative toxin biosynthetic cluster that are absent from other Pan isolates. One of these, HopH1, is present in other *P. syringae* pathovars. The other, HopZ2b, is a member of the YopJ class of effectors most closely related to those from *Acidovorax* and *Xanthomonas*. Putative host targets of these effectors are being sought using yeast two hybrid.

PS14-558

Transcriptional responses of *Pseudomonas syringae* to growth in epiphytic versus apoplastic leaf sites

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Pseudomonas syringae has a pronounced epiphytic phase that provides inoculum for infection and a pathogenic phase that involves multiplication in the leaf apoplast. To characterize the stimulons and traits contributing to growth in association with leaves, we performed whole-genome transcriptome profiling of *P. syringae* pv. *syringae* B728a cells recovered from the leaf surface and leaf apoplast, as well as cells exposed to in vitro treatments that reflect the predicted environmental conditions encountered on and in host plant leaves. The analysis indicated that exposure to leaf epiphytic and apoplastic sites induced transcriptomes that were more similar to those induced by osmotic stress and nitrogen starvation than by oxidative stress and iron starvation. Furthermore, greater transcript levels of genes involved in water deprivation tolerance in apoplastic sites suggested that, surprisingly, water limitation is more severe in the leaf than on leaf surfaces. A large number of transcripts responded differently to growth in epiphytic versus apoplastic sites. For example, the transcripts of genes involved in flagellar synthesis, chemotaxis and phenylalanine metabolism were induced primarily in epiphytic sites, suggesting movement prior to infection and degradation of phenylalanine, a precursor for phenylpropanoid-based defenses. In contrast, the transcripts of genes involved in the metabolism and transport of GABA, phytotoxins and syringolin were induced more within the leaves, supporting known roles in virulence as well as roles for syringolin in suppressing defenses beyond stomatal closure. These data are contributing to a coherent model of the adaptations of this widespread bacterial phytopathogen to distinct habitats within its host.

PS14-559

Identification and characterization of new type III effectors from *Xanthomonas campestris* pv. *vesicatoria*

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Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the type III-secretion (T3S) system which delivers effector proteins into the plant cell cytosol. In susceptible plants, type III effectors (T3E) interfere with host cell processes to the pathogen´s benefit. In resistant plants, however, recognition of individual effector proteins is mediated by plant resistance genes, often leading to the hypersensitive response, a fast and localized programmed cell death restricting pathogen ingress. Our lab studies the interaction between *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and its host plants pepper and tomato. We identified seven new T3E gene candidates in *Xcv* strain 85-10 based on homology of their gene products to known T3Es, the presence of eukaryotic motifs and conserved promoter elements suggesting co-regulation with the T3S system. RT-PCR analyses showed that expression of six candidate genes depends on the T3S system regulators HrpG and HrpX. The effector candidates were confirmed to be true type III effectors (Xops=*Xanthomonas* outer proteins) using translational fusions to the reporter protein AvrBs3Δ2 in type III secretion and translocation assays. Among the new T3Es, two Xops suppress plant defense responses and are crucial for bacterial virulence. We will discuss recent progress on the genetic and functional characterization of these T3Es.

PS14-560

XOO0635, a hybrid histidine kinase sensor of *Xanthomonas oryzae* pv. *oryzae*, is activated by sensing the low O_2 concentration and involved in stress tolerance and virulence

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The two-component signal transduction systems, which consist of a histidine kinase sensor and a response regulator, are dominant molecular mechanisms to monitor and respond to environmental stimuli in prokaryotes. Gene *XOO0635* in *Xanthomonas oryzae* pv. *oryzae*, a causal agent of bacterial leaf blight of rice, encodes a histidine kinase-response regulator hybrid protein harboring a PAS domain with a heme pocket. To investigate the functions of XOO0635, we generated a deletion mutant of the gene. When susceptible rice cultivar IR24 was inoculated with the mutant, the lesion lengths of the mutant were shorter than those of the wild type, suggesting that the gene is involved in bacterial virulence. Microarray assay revealed XOO0635-dependent up-regulation of XOO0131 (predicted protein required for attachment to host cells), XOO3715 (predicted osmotic shock protection protein) and XOO0635 itself along with other various genes including stress-responsive genes, which was confirmed by GUS reporter assay and semi-quantified RT-PCR (semi-qRT-PCR) analysis. The expression of XOO0131 and XOO3715 was increased under the low O_2 condition, suggesting that XOO0635 containing a heme pocket functions as an O_2 sensor. When we compared the stress tolerance between the XOO0635 mutant and the wild type, the mutant showed lower tolerant to osmotic (40% sorbitol), sodium (1 M NaCl) and H_2O_2 stresses than the wild type. These results suggest that XOO0635 is activated by sensing low O_2 concentration and involved in bacterial virulence by activating expression of stress-responsive genes.

PS14-561

The HSI-II gene cluster in *Pseudomonas syringae* pv. *tomato* DC3000 encodes a functional type VI secretion system, which is required for growth fitness in tomato and interbacterial competition

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Type VI secretion system (T6SS) in Gram negative bacteria was extensively studied during the past five years. In the model phytopathogenic bacteria, *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000, two gene clusters (named HSI-I and HSI-II) were identified using *in silico* analysis, but whether these two gene clusters encoding functional secretion systems is still uncertain. By using a GUS reporter system, we demonstrated that HSI-II and *hcp2* were expressed in both rich and minimal medium whereas HSI-I and *hcp1* were not. Expression of these T6SS-related genes is affected by the presence of different sugars in minimal medium. When *icmF2* and *clpV2*, two genes in HSI-II encoding core components, were deleted, secretion of Hcp2 was blocked, indicating that the HSI-II gene cluster encodes a functional secretion system. By means of a competitive index method, we successfully showed the virulence function of T6SS in *Pst*DC3000. More importantly, we found that interbacterial competition ability of *icmF2* and *clpV2* mutants was reduced when compared to wild type *Pst*DC3000. From the data of gene regulation and the biological functions we exhibited, T6SS in *Pst*DC3000 is likely to play an important role in ecological fitness.

PS14-562

Aconitase B is required for optimal growth of *Xanthomonas campestris* pv. *vesicatoria* on pepper leaves

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The aerobic phytopathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (Xcv) colonizes the intercellular spaces of pepper and tomato. Xcv probably has to cope with reactive oxygen species (ROS) and iron limitation, which potentially form part of the host defense response. An enzyme known to respond to ROS and iron limitation and that might contribute to the successful proliferation of Xcv in the host is the iron-sulfur [Fe-S] protein aconitase, which converts citrate to isocitrate. Xcv contains three putative aconitases, two of which belong to the AcnA class and the other is an AcnB enzyme. *In vitro* growth of *acnB* mutants in shake-cultures was like wild type, whereas *in planta* growth and disease symptom formation on pepper plants were both delayed in the *acnB* mutant. The *acnB* mutant also showed enhanced susceptibility towards the superoxide-generating chemical menadione. Moreover, when the *acnB* mutant was grown with citrate as carbon source, it grew more poorly than the wild-type confirming that AcnB has a role in citrate utilization in Xcv. The *acnB* gene is co-transcribed with two upstream genes XCV1925 and XCV1926, which are predicted to encode a toxin/antitoxin system. An Xcv mutant lacking both XCV1925 and XCV1926 exhibited strong up-regulation of AcnB. Thus, we propose that optimal growth and survival of Xcv in pepper plants depends on AcnB and that the levels of AcnB appear to be tightly controlled by a toxin/antitoxin system. The signal to which this regulatory system responds is currently unclear, but possibilities include citrate, iron or oxidative stress.

PS14-563

Identification of genes involved in *Ralstonia solanacearum* phage infection and LPS biogenesis

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Ralstonia solanacearum causes a deadly wilting disease on a broad range of crops. However, information on the determinants involved in biogenesis of its lipopolysaccharides (LPS), which plays crucial roles for bacterial fitness in various environments and interaction with phages, was unavailable. In this study, we screened for phage-resistant *R. solanacearum* mutants, aiming to identify key components involved in phage infection and LPS biogenesis. In addition to conserved proteins involved in lipid A and inner core synthesis, we identified a group of new LPS-biosynthesis loci whose homologs are absent in *E. coli*. By characterizing the mutants, our results revealed that *R. solanacearum* R-LPS synthesis is sufficient to maintain membrane integrity and cause local disease response in *Nicotiana benthamiana*, while S-LPS production is a determinant for phage adsorption, resistance to polymyxin B and effective *in planta* proliferation. Moreover, disruption of loci putatively involved in phospholipid trafficking and peptidoglycan recycling enables the bacterium resistant to the phage, probably by obstructing phage adsorption and DNA injection, respectively. Comparative sequence analysis showed conservation of some of these loci among representative Gram-negative bacteria, implying their common functions. This study is the first to extensively decipher genetic nature of *R. solanacearum* LPS biogenesis, and provides new insights into *R. solanacearum*-phage interaction.

PS14-564

The *Clavibacter michiganensis* subsp. *michiganensis*-tomato interactome reveals perception of pathogen by host and suggests mechanisms of infection

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The Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the causal agent of wilt and canker disease of tomato (*Solanum lycopersicum*). Mechanisms of *Cmm* pathogenicity and tomato response to *Cmm* infection are not well understood. To explore the interaction between *Cmm* and tomato, multi-dimensional protein identification technology (MudPIT) and mass spectrometry were used to analyze *in vitro* and *in planta* generated samples. The results show that during infection *Cmm* senses the plant environment, transmits signals, induces and then secretes multiple hydrolytic enzymes, including several families of serine proteases, glycosyl hydrolases, and other plant cell-wall degrading enzymes. We identified and further characterized two *Cmm* pathogenicity specific putative transcriptional regulators, and show that their deletion affects expression of *Cmm* virulence factors. Tomato induction of pathogenesis-related (PR) proteins, LOX1, and other defense-related proteins during infection indicates that the plant senses the invading bacterium and mounts a basal defense response, although partial with some suppressed components, including class-III peroxidases and a secreted serine peptidase. The tomato ethylene-synthesizing enzyme ACC-oxidase was induced during infection with the wild-type *Cmm*, but not during infection with an endophytic *Cmm* strain, identifying *Cmm*-triggered host-synthesis of ethylene as an important factor in disease symptom development.

PS14-565

Molecular characterization of AvrBs3 from *Xanthomonas*

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Xanthomonas campestris pv. *vesicatoria* is the causal agent of bacterial spot disease on pepper and tomato plants. Essential for pathogenicity is the type-III-secretion (T3S) system, which translocates effector proteins into the cytoplasm of the plant cell. One well-studied effector is AvrBs3, the founding member of the large transcription activator-like (TAL) effector family. AvrBs3 acts as a transcriptional activator in the plant cell and upregulates *UPA*

genes e.g. the resistance gene *Bs3* and *UPA20*. Expression of *Bs3* induces the hypersensitive response (HR) whereas *UPA20* induction leads to hypertrophy, a cell enlargement on susceptible pepper plants. AvrBs3 contains a T3S secretion and translocation signal in the N-terminal region, two functional nuclear localisation signals and an acidic activation domain in the C-terminal region. DNA binding is mediated by a central repeat domain which is composed of 17.5 repeats of a nearly identical 34 amino acid motif. The repeat region is a novel DNA-binding fold. Recent studies revealed that one repeat specifically binds one base pair in the target promoter DNA. Specificity is determined by the variable residues at position 12 and 13 of each repeat, termed repeat-variable diresidue (RVD). Besides DNA binding the repeat region is also required for self-interaction of AvrBs3, which occurs *in vitro* and *in vivo*. Here, we show that self-interaction is affected by mutations in the non-RVDs of the repeat region. Our data reveal the underlying mechanism for AvrBs3 self-interaction and its influence on DNA binding and gene induction.

PS14-566

Arabidopsis thaliana as an experimental host for *Xylella fastidiosa*, causal agent of citrus variegated clorosis

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Citrus variegated clorosis (CVC) is one of the most severe sweet orange disease, that leads to significant losses to the citrus industry in Brazil. It is caused by the xylem-restricted gram negative bacteria *Xylella fastidiosa*. Although breeding approaches be a strategy to obtain tolerant or resistant genotypes, transgenic lines with genes from resistant species of citrus or even from the bacteria (Pathogen Derived Resistance) have been used. However, getting transgenic orange plants takes long time, because of their long juvenile period and low transformation efficiency. Therefore, it is recommended that all candidate genes should be previously validated. To accelerate the evaluation of these genes, *Arabidopsis thaliana* has been tested in its ability to be infected by *X. fastidiosa*, for future CVC resistance candidate genes evaluation. We challenged Col-0, Van-0 and Tsu-1 ecotypes with a GFP-transformed *X. fastidiosa* strain 11399. Five weeks old plants were inoculated with a 4,6.10$_7$ CFU bacteria solution. After 14 days, GFP-microscopy and qRT-PCR detection of *X. fastidiosa* were performed. Bacteria were detected in all ecotypes evaluated by GFP-microscopy. Col-0 plants had higher bacteria titers than Tsu-1 and Van-0. Compared with previously published data, these two ecotypes were more susceptible than Col-0 infected with *X. fastidiosa* causing Pierce disease, suggesting that different responses may occur among Arabidopsis ecotypes and *X. fastidiosa* strains. These results point out that *A. thaliana* can be used as model plant to evaluate plant-bacteria interactions, but *A. thaliana* ecotypes and *X. fastidiosa* strains should be previously tested for suitable better compatibility.

PS14-567

Adaptation of *Pectobacterium atrosepticum* SCRI1043: to survive in order to infect.

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Bacterial populations are highly integrated holistic communities, consisting of physiologically and morphologically heterogeneous cells. This allows microorganisms to adapt flexibly to different environmental conditions and persist in prolonged stress. The adaptation of pathogenic microorganisms to starvation is required during the stage of the life cycle which takes place outside the host organism. We studied the adaptive reactions of plant pathogenic bacterium *Pectobacterium atrosepticum* SCRI1043 (*Pba*) during carbon and phosphorus starvation. Bacteria implemented different adaptive programs, depending on the physiological state of the cultures and inoculation titer. At high initial titer (10^8 CFU/ml) the number of colony-forming units decreased due to the processes of the autolysis and the formation of dormant cells (Gorshkov et al., 2009). Ultrastructural modifications of cells took place: the cells with intracellular polyhydroxyalkanoate granules, and the cells with friable polysaccharide capsules were observed. But at low initial titer (10^3 CFU/ml) cell division coupled with the formation of particular cell morphotype with condensed nucleoid and augmented periplasmatic space occurred. Thus, the initial stage of the response to starvation was stabilization of the cell density in the range of 10^6-10^7 CFU/ml. as a result the bacterial populations resistant to high temperature, oxidative stress and rifampin were formed. Starving bacteria retained the ability to subsequently infect the host plant. Thus, regardless of the adaptation tactics the strategic result of the processes occurring in starving cultures of *Pba* was the formation of specialized bacterial phenotypes, which were capable of persistence during the unfavorable conditions and infecting a host plant.

PS14-568

Diversity of HrpL regulons in *Pseudomonas syringae* isolates

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Pseudomonas syringae (*Ps*) is a Gram-negative bacterial plant pathogen with high phylogenic diversity responsible for disease on many crop species. HrpL is the master regulatory transcription factor of *Ps* that controls expression of the genes encoding components of the type-III-secretion system, essential for virulence, and expression of the type-III-secreted effectors (T3Es). HrpL also regulates expression of genes encoding "non-effector" proteins including toxin producing enzymes and proteins not previously associated with virulence. We implemented and refined transcriptional analysis methods using cDNA and high-throughput sequencing data to identify HrpL-regulated genes for four isolates of *Ps* using either complete or draft genomes. The quality of our methods was confirmed using the well-studied pathogen, *Pto*DC3000, along with real-time RT-PCR. Comparative analysis of the HrpL-regulon across *Pto*DC3000, *Pph*1448a, *Psy*B728a and *Por*1_6 defined strain-specific variability for not only T3Es but also for genes encoding non-effector proteins. *Psy*B728 has the smallest HrpL-regulon, encoding not only fewer T3Es than other strains but also fewer non-effector proteins. Variation in the number of non-effector genes in the HrpL-regulons of our strains was not only due to the presence/absence of genes, but also a result of group-specific *hrp* box mutations, or variability in upstream regions. We demonstrated the virulence function of several non-effector genes by testing the growth of mutants *in planta*. We highlight the advantages of next generation transcriptomics to identify putative virulence factors and their recruitment into and out of the HrpL regulon across the *Ps* phylogeny by integrating genomic, transcriptomic, and phylogenetic information to gain insight into pathogenicity.

PS14-569

Differential expression of SU91-linked QTL in the interactions between common bean and strains of common bacterial blight pathogens

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Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Xcp) and *Xanthomonas fuscans* subsp. *fuscans* (Xff), is a damaging disease of common bean (*Phaseolus vulgaris* L.) throughout the world. Two Xcp strains (nos. 18 and 98) and two Xff strains (nos. 12 and 118), collected locally, showed differential virulence on a set of bean genotypes. Ninety F4:5 recombinant inbred lines (RILs) derived from a cross between susceptible Sanilac and resistant OAC 09-3 were phenotyped in an artificially inoculated field disease nursery and in the growth room using a mixture of those four strains as well as using single strains (either no. 98 or no. 118), which were highly pathogenic. The RILs were genotyped using molecular marker SU91, known to be associated with a major CBB resistance QTL. SU91 accounted for 30 to 40 % of phenotypic variations when the RIL population was inoculated with a mixture of four strains, but only 5 to 10% of variations when inoculated with a single strain no. 98 or no. 118. These results suggested the existence of interactions between different pathogen strains, or between pathogen strain and resistance QTL. Results highlight the importance of selecting appropriate pathogen strains for CBB screening in bean breeding programs and emphasize the need for selection of genotypes with resistance to multiple strains of the pathogen.

PS14-570

Initial characterization of the two type VI secretion systems in *Pseudomonas syringae* pathovar *tomato* DC3000

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Pseudomonas syringae pathovar *tomato* strain DC3000 causes disease in plants by secreting effector proteins and the phytotoxin coronatine. Effectors, which are injected into plant cells by a type III secretion system (T3SS), inhibit plant defenses in susceptible hosts and allow bacterial colonization of the leaf interior. Besides for the T3SS, other protein secretion systems in DC3000 may contribute to plant infection. Here, we examined the roles of two putative type VI secretion systems (T6SS-I and T6SS-II) in DC3000-plant interactions. We show that T6SS-II genes are highly expressed in minimal medium, whereas T6SS-I genes are transcribed at lower levels. We also constructed DC3000 strains containing large deletions in the T6SS-I and T6SS-II loci. Our results show that the T6SS-II locus is required for optimal DC3000 growth and virulence in tomato and Arabidopsis. T6SS-II is additionally required for DC3000 suppression of callose deposition in Arabidopsis. The T6SS-II mutation did not affect T3SS or coronatine function, as it did not alter the ability of DC3000 to cause the hypersensitive response, induce chlorotic rings around disease lesions, or express the effector gene *avrPto*. Overall, our results indicate that the T6SS-II mutation in DC3000 may affect secretion of proteins that suppress plant defenses. However, T6SS deletion mutants also aggregate in static cultures and form denser biofilms on plastic. Therefore, the T6SS-II mutation could indirectly affect DC3000 survival in plants by causing increased bacterial aggregation. Current work is focused on characterizing the effects of mutations in individual T6SS genes on plant infection and bacterial aggregation.

PS14-571

Relationship between molecular diversity and HR induction in tobacco of Japanese strains of *Ralstonia solanacearum*

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Ralstonia solanacearum is a species complex with exceptional diversity amongst strains from different hosts and geographical origins. The genetic diversity of 120 *R. solanacearum* strains isolated from a variety of host plants across Japan was assessed on the basis of hypersensitive response (HR) in tobacco leaves and phylogenetic analyses of endoglucanase gene *egl*, *hrpB*, and *gyrB*. Phylogenetic analysis of *egl* revealed that only three strains belonged to phylotype IV, and 117 strains belonged to phylotype I. Partial sequences of HrpB were identical among phylotype I strains except for one strain. Analyses using the partial nucleotide sequences of the *gyrB* and *egl* gene fragments grouped phylotype I strains into 11 *gyrB* and 8 *egl* types, respectively, whereas analyses using the partial amino acid sequences of GyrB and Egl grouped phylotype I strains into 4 GyrB and 5 Egl types, respectively. Dendrograms based on GyrB and Egl differ each other, indicating that housekeeping genes and virulence-related genes have evolved independently. Biovars of Japanese isolates did not seem to be related to genetic diversity. Using multilocus sequence typing of GyrB and Egl, we identified 10 unique sequence types within the Japanese phylotype I strains. Strains belonging to the GyrB42 or GyrB66 type caused wilt in tobacco, and strains belonging to GyrB2 or GyrB9 type elicited HR, demonstrating that HR induction in tobacco is genetically differentiated in the Japanese strains of *R. solanacearum*.

PS14-572

Agrobacterium tumefaciens 6b gene on T-DNA has activity of histone chaperon and represses expression of auxin-response genes in Arabidopsis

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Agrobacterium tumefaciens harboring a Ti plasmid is a causative agent for crown gall tumors. A gene, *6b*, encoded by T-DNA in the Ti plasmid has been reported to have a role in host range determination of Agrobacteria. The 6b stimulates the plant hormone-independent division of cells and cause morphological abnormalities. We showed that 6b interacts with tobacco nuclear proteins (NtSIP1 and NtSIP2) and histone H3, and has a histone chaperone activity, suggesting that 6b might affect chromatin structures. It is also reported that 6b disturbs miRNA pathway. These data suggested that 6b might affect expression of various genes that are involved in cell proliferation and plant morphology. A role of 6b in the gene expression in the plants, however, remains to be determined. Here we report the results of microarray and real-time RT-PCR analyses with Arabidopsis plants overexpressing the *6b* gene. The transcript levels of genes involving in cell differentiation and proliferation were altered in *6b*-trasgenic plants. Interestingly, transcript levels of several auxin-inducible genes were decreased in *6b*-tragsgenic Arabidopsis. Our results also showed that *6b*-transgenic Arabidopsis plants were insensitive to auxin, similarly to the *bodenloss* mutant.

PS14-573

Diversity in *Erwinia amylovora* virulence on different *Malus* cultivars

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Terminal shoots of one-year old apple trees cvs. Idared, Elstar and Quinte grown on M9 rootstock in greenhouse were inoculated with 8 *Erwinia amylovora* strains. Six of them were isolated from different hosts in Poland while other two originated from *Malus* in the USA. Virulence of tested strains was expressed as a percentage of shoot necrosis in relation to entire length of shoot measured

six weeks after inoculation. Cultivar Elstar appeared to be most susceptible to all strains while Idared showed differential reaction depending on strain used. The lowest virulence was shown by two strains isolated from *Crataegus* - 52.6 and 62% while other strains caused over 75.6% shoot necrosis. The highest differentiation in disease development to tested strains was observed on Quinte. The largest necroses were produced by both strains from the USA (84.1 and 89.4%, respectively) whereas other strains produced only little necroses including strain E2 (from *Crataegus*) which was not pathogenic to this cultivar. The highest diversity between virulence of all strains was observed on Quinte. Study on genetic diversity of *E. amylovora* strains including sequence analysis of genes involved in pathogenicity process and RAPD tests showed their high similarity. The only differences were proved using AFLP analysis with two sets of restriction enzymes *Eco*RI + *Pst*I and *Eco*RI + *Mse*I and 6 out of 11 selective primers. Strains originating from USA grouped together but no correlation between AFLP patterns, geographical origin, host plant or virulence was found.

PS14-574

Two avirulence effector genes of Japanese *Ralstonia solanacearum* strains are involved in pathogenicity to tobacco

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Infection of phylotype-I strains of gram-negative pathogen *Ralstonia solanacearum* on tobacco plants results in different disease symptoms. While a wild type strain GMI1000 isolated from South America elicits HR, the mutant lacking two effector genes, *avrA* and *popP1*, causes tobacco plants to wilt. We investigated the involvement of two effector genes of Japanese strains in pathogenicity to tobacco. We used two virulent strains, OE1-1 and MAFF241653, and four HR-eliciting strains, 8107, MAFF211471, MAFF211496, and MAFF301520. There are two types *avrA* sequences, a GMI1000-type and an RS1000-type, in Japanese strains. Both AvrAs are 59% identical in amino acid sequences. While MAFF211496 carries the GMI1000-type *avrA*, other five strains have the RS1000-type *avrA*. Three strains, 8107, MAFF211471, and MAFF241653, contain *popP1*, however, other three strains have no *popP1* gene. We deleted *avrA* and/or *popP1* genes from HR-eliciting strains. All the mutants, no matter which type of *avrA* gene was deleted, still elicited HR. When *popP1* gene was transferred into the virulent strain OE1-1, the transformant strain significantly reduced the virulence on tobacco. These indicate that *avrA* and *popP1* of Japanese strains have different effects on tobacco wilt incident from those of GMI1000.

PS14-575

The role of *hrpRS* in regulation of virulence gene expression in *Pseudomonas syringae* pathovar tomato DC3000

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Pseudomonas syringae utilizes a type III secretion system (T3SS) to translocate virulence proteins called effectors directly into plant cells. In order to deploy the T3SS at appropriate times, T3SS gene expression is tightly controlled by a complex network of transcription factors. The alternative sigma factor HrpL directly regulates expression of genes encoding secreted effectors and structural components of the secretion apparatus. In turn, *hrpL* transcription is directly activated by two members of the bacterial enhancer binding protein family, HrpR and HrpS. Our goal is to better understand how expression of the *hrpRS* operon is controlled, and how HrpR and HrpS regulate *P. syringae* pathovar *tomato* DC3000 virulence. To this end, *hrpRS* expression was measured during DC3000 growth in various media and environmental conditions. We found that *hrpRS* expression was not induced as highly as *hrpL* in all conditions tested. To examine HrpR/HrpS activation of *hrpL*, *lacZ* reporter plasmids containing various lengths of the *hrpL* promoter region were constructed. HrpR/HrpS activated *hrpL* transcription via enhancer sequences significantly upstream of the *hrpL* promoter in both *E. coli* and *P. syringae*. Current studies are focused on identifying the HrpR/HrpS binding site(s) upstream of *hrpL*. To this end, we are carrying out DNA binding assays *in vitro* with the *hrpL* promoter region and purified HrpR and HrpS proteins. Overall, these studies will contribute to a better understanding of virulence gene regulation in *P. syringae* and aid in identification of genes (besides for *hrpL*) that are directly regulated by HrpR/HrpS.

PS14-576

Differential expression of *in vivo* and *in vitro* protein profile of outer membrane of *Acidovorax avenae* subsp. *avenae*

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In this work, we examined and compared the expression of OM proteins of rice pathogen *Acidovorax avenae* subsp. *avenae* strain RS-1, a Gram negative bacterium, both *in vitro* culture medium and *in vivo* rice plants. Global proteomic profiling of *A. avenae* subsp. *avenae* strain RS-1 comparing *in vivo* vs. *in vitro* revealed differential expression of proteins geared towards survival and pathogenicity of the rice pathogen in host plants. The shotgun proteomics analysis of OM proteins resulted in identification of 97 proteins *in vitro* and 64 proteins *in vivo* by mass spectrometry. Among these OM proteins, there were a high number of porins, TonB-dependent receptors, lipoproteins of the NodT family, ABC transporters, flagellins, and hypothetical proteins in both conditions. However, the major proteins such as phospholipase and OmpA proteins are expressed *in vitro*, while the surface anchored protein F, ATP-dependent Clp protease, OmpA/MotB domain containing proteins are expressed *in vivo*, indicating that these *in vivo* OM proteins may have role in the pathogenicity of *A. avenae* subsp. *avenae* strain RS-1. In addition, the LC/MS identification of OmpA and MotB validated the *in silico* prediction of Type VI secretion system core components based on the genome wide analysis of *A. avenae* subsp. *avenae* strain RS-1. To the best of our knowledge, this is the first study revealing the *in vitro* and *in vivo* protein profiling in combination with LC/MS Mass spectra, *in silico* OM proteome and *in silico* genome wide analysis of pathogenicity or plant host required proteins of plant pathogenic bacteria.

PS15-577

CFGP 2.0: A standard web-based bioinformatics portal for comparative and evolutionary genomics

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Currently more than 200 fungal genomes have been sequenced, presenting the largest number of genomes in the eukaryotic domain. With the availability of increasing sequenced fungal genomes aided by Next-Generation Sequencing technologies, fungal genomes could be one standard subject for comparative and evolutionary bioinformatics. A standardized data warehouse is required to compromise heterogeneous formats of genome sequences and to enable the comparative studies. As no sequence repositories focused on the fungi, archiving all the available fungal

genomes and developing standardized platform had immediate importance. As one solution, Comparative Fungal Genomics Platform (CFGP; http://cfgp.snu.ac.kr/) was released in 2007 aiming for the comprehensive bioinformatics workbench with the standardized data warehouse. Now the CFGP embraces 284 fungal genomes from 152 different species, 39 from plants and 105 from the kingdom Metazoa. To provide easy-to-use and efficient way to manage and analyse sequence data, the CFGP offers a data analysis hub, called Favorite. The Favorite provides virtual storage for sequences collected by users and 27 bioinformatics tools for direct analysis. Furthermore, the Favorite is implemented in Fungal Transcription Factor Database (http://ftfd.snu.ac.kr/), Fungal Cytochrome P450 Database (http://p450.riceblast.snu.ac.kr/) and Fungal Secretome Database (http://fsd.snu.ac.kr/), synced in real-time with the CFGP. Collectively with the standardized data with bioinformatics tools expanded with diverse secondary databases, the CFGP 2.0 can be a representative platform for comparative genomics and phylogenomic researches in the eukaryotic domain.

PS15-578

Laboratory Information Management System for functional genomics of *Magnaporthe oryzae*

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A Laboratory Information Management System (LIMS) is a computer software for the management of all laboratory information and instruments using online database. With rapid accumulation of genome information, experimental data are being generated at genome-wide scale. However, majority of datasets was not comparable because there is no globally accepted standardized experimental protocols and data acquisition formats. Although much progress has been accomplished in plant and animal genomics studies, LIMS has not been implemented in the field of functional genomics studies in fungal biology and molecular plant-microbe interaction. LIMS was designed for *Magnaporthe oryzae*, the rice blast pathogen, to provide not only integrated management system for functional genomics research, but also standardized experimental protocols that have guidelines for phenotype assays and data acquisition formats. As the first step, we collected all experimental protocols and data acquisition methods used for phenotype characterization from published research papers,compared and analyzed for each specific assays, and proposed the standard guidelines for each phenotype assays. To develop a web-based management system, work processes are divided by time-dependent/independent processes for generating gene replacement mutants and phenotype assays. All data generated from each experimental step can be stored and comparatively analyzed in LIMS. LIMS will provide new paradigm for functional genomics and phenomics of molecular plant-microbe interaction research.

PS15-579

KNApSAcK Family Databases connect biological activities of metabolites and plants with microorganisms

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Various mutually/unilaterally beneficial/harmful relationships between plants and microorganisms affect plant health such as disease control and symbiotic nitrogen fixation. Systematic analysis of enormous numbers of plant-microorganism interactions is helpful to comprehensively understand the agricultural productivity and the environmental preservation using information derived from plant and microorganism -omics. To attain this purpose, we developed KNApSAcK Family databases (DBs). In the present study, we introduce the DBs focused on the plant-microorganism relationship. The KNApSAcK Family consists of the Metabolomics DB system (KNApSAcK Core, contains 101,500 species-metabolite relationships encompassing 20,741 species and 50,048 metabolites) and the Multifaceted Plant Usage DB including (i) KNApSAcK WorldMap, 41,548 geographic zone-plant pair entries emcompassing 222 geographic zones; (ii) KAMPO, 336 formula names for Kampo in Japan encompassing 278 plants; (iii) JAMU, 5,310 formulas for Jamu in Indonesia encompassing 550 plants; (iv) Natural Activity, 2,418 biological activities and 33,706 pairwise relationships between plants and their biological activities; and (v) Metabolite Activity, 2,087 biological activities and 5,043 pairwise relationships between metabolites and their biological activities. In the Natural/Metabolite Activity DBs, users can retrieve plants or metabolites by inputting a search term pertaining to biological activity, including biochemical activities and diseases in a text box. All of the data regarding biological activities are linked to relevant citations from the literature. Thus, using these integrated platforms, we can easily generate systems-level information, beginning with the identification of the plants/microorganisms and progressing through the biological activities, to address the interaction effects. The KNApSAcK family can be accessed freely via the website http://kanaya.naist.jp/KNApSAcK_Family/.

PS15-580

Systems biology approach to study potato PVY interaction

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Potato virus Y (PVY) is a severe plant pathogen responsible for yearly losses in production of Solanaceae crops worldwide. Plant responses to viruses and the disease development are different and much less explored in comparison to bacterial or fungal infections. In single component studies the complexity of the plant pathogen interaction at molecular level can lead to limited conclusions that may fail to notice important changes in physiological processes. Omics approaches in combination with modelling, offer a more holistic view of the processes are therefore a major step forward in understand these interactions. In our studies, gene expression in the disease response of the susceptible, tolerant and resistant potato (*Solanum tuberosum* L.) cultivars to PVY infection was investigated at different times after infection, using transcriptomics approaches, among them subtractive hybridization, cDNA microarrays and real-time PCR. We are additionally combining the results on transcriptome level with proteome and metabolome profiling. Most pronounced is the regulation of photosynthesis-related genes expression, expression of genes involved in sugar metabolism and redox state maintenance as well as regulation of several defense signalling related genes. Dynamics of selected gene expression was significantly different if observing sensitive, tolerant or resistant type of interaction. Our results show that not only the components involved but also the timing and intensity of response are extremely important for the outcome of plant virus interaction.To enable better understading of the system under investigation a model of potato PVY interaction signalling was built and results of simulations compared to experimental data.

PS15-581

Codon usage pattern of predicted operon-like genes in *Arabidopsis thaliana*

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Operon-like genes in eukaryotes are defined by co-regulation and neighborhood position in genome. It is known that operon-like gene clusters implicate several secondary metabolic pathways in plants. Genome-wide prediction of operon-like gene clusters should contribute to functional annotation efforts and might provide novel insight into gene expression system aspects. Initially, we predicted the co-expressed neighborhood gene clusters by a statistical method based on 1469 microarray dataset of *Arabodipsis thaliana*. As a result, we predicted 34 operon-like gene clusters each including 3 to 22 genes. Further, we estimated annotation of genes for functional relationship analysis. We found that a number of operon-like gene cluster candidates are associated with metabolism, containing P450 genes restricted to the Brassia family and predicted to be involved in secondary metabolisms. Interestingly, several operon-like clusters tend to contain ribosomal genes. These observations suggest that neighborhood genes encode proteins involved in the same biological processes. To investigate the expression systems of the gene clusters, we performed an analysis of codon-usage bias. Some operon-like genes have a different codon pattern from the other genes in the same cluster. It may be suggested that those heterogeneity of codon usage of genes reflects efficacy of translation which partitions the operon-like clusters.

PS15-582

Stochastic simulation of metabolic network of *Arabidopsis thaliana* using experimental data

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Predictive simulations of the metabolites using experimental data are complicated because the metabolic network itself is complex and huge. In addition, even if experimental data are obtained, as the absolute amount of the metabolites cannot be obtained. To attain this situation, we developed a method of stochastic simulation for prediction of the amount of metabolites using the experimental data. The relative amounts of the 269 metabolites in *Arabidopsis thaliana* were measured with a mass spectrometry. First, two durations of the experiments were selected by multivariate analysis, where the amount of metabolites changed remarkably. Then a product that increased the most and 10 substrates that decreased the most in the experiments in each duration were selected. Next, the shortest pathways and related metabolites from the substrates to the product were searched from the Aracyc database. Using these pathways, we made a series of stochastic simulations setting initial value to 0 for the product. The simulation results show that almost all of the substrates decreased and the product increased with time as it was expected according to the experimental results. The metabolites classified in the same group by a hierarchical clustering method tended to have similar time series profiles. These results indicate that this simulation makes it possible to reproduce the trends of the time series of the experimental data.

PS15-583

Development of the micro-particle transportation system using photorepellent response in apo-symbiotic green paramecia

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Recently, the areas of micro-robotic studies have been expanded to cover the use of living microorganisms as novel target materials controllable within the micro-sized systems. We employed the cells of green paramecia (*Paramecium bursaria*) as a working model for micro-robotic study. Naturally, green paramecia can be found in fresh water environments such as rivers, ponds, and lakes. The green paramecia can swim through ciliary movements based on the action of motor proteins precisely controlled under cellular signaling events. Cells of apo-symbiotic green paramecia may fulfill two key criteria to be used as the electrically controllable micro-particle carriers. Firstly, the materials have a capacity for foreign nano- and micro-sized particles by replacing the intracellular space for endogenously growing symbiotic green algal cells. Secondly, the apo-symbiotically conditioned cells can migrate towards the darkness when exposed strong light stimulus, whereas the wild-type cells of green paramecia with symbiotic algae often favor the illuminated conditions. This type of cellular behavior is known as photorepellent response. Taken together, the conditioned apo-symbiotic cells of green paramecia can be used as moving capsules designed to contain any particles of interests, controlled under light stimuli. Here, we report on our novel approach on photo-driven micro-particle transportation using green paramecia.

PS15-584

Gene discovery of *Colletotrichum acutatum* - strawberry interaction

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The fungal pathogen *Colletotrichum acutatum* has a wide host range and causes diseases and severe yield losses worldwide on economically important fruit crops. Several secondary metabolites have been identified during the necrotrophic development of *Colletotrichum* spp., but only few have shown phytotoxic activity. *Colletotrichum* spp. also produces a range of hydrolytic enzymes that play a role in the infection biology. The aim of this project is to screen for enzyme activity and to investigate differentially expressed genes of importance in the strawberry - *C. acutatum* interaction. Flask cultures with liquid strawberry media were inoculated with *C. acutatum*. Microscopy, pH-measurements and enzyme activities were monitored in a time course experiment. The enzyme activity was determined using (AZCL)-polysaccharide plates. Fungal secondary metabolites were studied by GC-MS analysis. *C. acutatum* produced casein, collagen, arabinoxylan and β-glucan degrading enzymes in flask cultures. The pH increased rapidly during culture growth. Two time points of interest based on pH and enzyme activity were selected for RNA isolation and subsequent SSH to identify differentially expressed genes. Both fungal house keeping genes and genes encoding proteins involved in the biosynthetic pathways of secondary metabolites were identified. Selected genes will be further characterised by genome walking, heterologous expression and qRT-PCR. Several interesting fungal secondary metabolites were also identified and more biochemical analyses will be carried out. In addition, transcriptome analysis is

going to be performed in future. The importance of the identified genes and metabolites will be further studied during *C. acutatum* infection of strawberry fruits.

PS18-585

Infection pattern and growth promotion effects between *Burkholderia cepacia* and *Zea mays*

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Bacteria are common inhabitants of the rhizosphere and the internal tissues of plants. Amongst, *Burkholderia* is a genus rich in plant-associated nitrogen fixers and with phosphate solubilizing abilities. Phosphate solubilizing bacteria (PSB) has found many usages in agriculture, including enhancements of crop growth, crop yield, and crop disease-resistance. Recently, laboratory experiments have discovered that a Taiwan native PSB, *Burkholderia cepacia*, can solubilize Ca-, Fe-, and Al-bound phosphates and, more interestingly, this genus of bacteria also acts as an endophyte. At present, little is known with regard to the infection pattern of this particular microbium and its beneficial effects on crops. Our study revealed that *B. cepacia* can only infect maize during the seed germination stage and persist as an endophyte over the period of time of our analysis. Maize seedlings infected of *B. cepacia* showed a significant 23% increase in plant height and other parameters. Nutrient analysis also showed an increased value of N and P elements in infected maize seedlings as compared to the non-infected controls. Utilization efficiency of P in the culturing medium was also increased in seedlings infected of *B. cepacia*. On the contrary, *B. cepacia* does not establish endophytic relationship with rice. In summary, these preliminary results allow us to identify local beneficial microbes that merit further field tests.

PS18-586

Rhizophere signal strigolactone produced by plant cell cultures

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Root parasitic plants and arbuscular mycorrhizal fungi receive strigolactones (SLs) as host recognition signals in the rhizosphere. In host plants, SLs play a key role in the regulation of shoot branching. Despite their important functions, the biosynthesis pathway of SLs has not been fully elucidated. As it is in general difficult to characterize endogenous SLs in plants since their levels are extremely low, we attempted to analyze endogenous SLs using plant cell cultures as a tool for study on endogenous SLs. Suspension cell cultures of Arabidopsis and rice were provided from RIKEN BioResource Center of Japan. The suspension cell cultures were separated into cells and culture media and extracted with ethyl acetate. The neutral ethyl acetate-soluble fractions were examined by a bioassay using seeds of root parasitic plant Orobanche minor. Remarkable activities on the germination stimulation were found in both the cells and culture media. It was shown by LC-MS/MS that both the cells produced SLs including orobanchol, orobanchyl acetate and 7-hydroxyorobanchyl acetate, and also released these SLs to the culture media. Furthermore, we investigated the effect on the SL exudation by nutrient conditions. When the cultured cells were grown in a phosphate-deficient medium, the exudation of SLs into culture media was promoted. This finding indicates that plant cell cultures would be a good tool for understanding the exudation mechanism of SLs in the rhizophere.

PS18-587

***PrCYP707A1*, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analog GR24**

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Seed dormancy in obligate root-parasitic plants is released, following a conditioning period, by a chemical stimulus secreted by the roots of host plants. Using *Phelipanche ramosa* as model, we demonstrated that seeds required a conditioning duration of at least four days to be receptive to the synthetic germination stimulant GR24. By applying a cDNA-AFLP procedure on the seeds, we isolated 58 Transcript Derived Fragments (TDF) showing change in their expression pattern upon GR24 treatment. Among the isolated TDFs, two up-regulated sequences corresponded to an ABA-catabolic gene, *PrCYP707A1*, encoding an abscisic acid 8-hydroxylase. Using RACE experiments, two full-length cDNAs, *PrCYP707A1* and *PrCYP707A2*, were isolated from the seeds. Both genes were constantly low expressed during conditioning while a first decline in ABA level was registered. After conditioning, GR24 application triggered a strong *PrCYP707A1* up-regulation during the first 18h followed by a second 8-fold decrease in ABA level detectable 3 days after treatment. *In situ* hybridization experiments on GR24-treated seeds revealed a specific *PrCYP707A1* mRNA accumulation in the perisperm cells beneath the micropyle. The inhibitory activity on seed germination of Abz-E2B, a specific inhibitor of CYP707A enzymes, was demonstrated as a non-competitive antagonist of GR24 with a reversible inhibitory activity. Those findings demonstrate that *P. ramosa* seed dormancy breakdown relies on an ABA catabolism mediated by a GR24-dependent activation of *PrCYP707A1*. These also corroborated previous studies on the putative location of germination stimulant receptors in perisperm cells of seeds.

PS18-588

The phloem network in the parasitic plant *Phelipanche ramosa*; carboxyfluorescein labelling and characterization of three sucrose transporteurs

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Following attachment to host roots, broomrapes (*Orobanche spp* and *Phelipanche spp*) develop a tubercle, then a subterranean shoot that flowers after emergence from the soil. Due to the achlorophyllous nature of broomrapes, sucrose uptake from the host phloem supports the parasite growth. By using a non permeant fluorescent tracer of symplasmic phloem connexions (carboxyfluorescein), the occurrence of direct phloem connexions at the host-parasite interface was demonstrated. Besides, phloem network organization and phloem unloading inside the parasitic plant need clarification. By applying carboxyfluorescein to host leaves (rapeseed and tomato), we observed that, except the adventitious root apices of young tubercles, all the sink areas of the parasitic plant *P. ramosa*, including tubercle parenchyma, shoot apical meristems and shoot axillary buds are symplastically isolated from the parasitic phloem network. Consequently, an

active apoplastic unloading of the host-derived sucrose should occur in those sink areas. In this context, three sucrose transporter cDNA were isolated from *P. ramosa* (*PrSUT*). While *PrSUT2* expression was low overall parasite development, *PrSUT1* was by far the more expressed *PrSUT* gene in all the organs and *in situ* hybridization experiments emphasized transcript accumulation specifically in phloem cells. PrSUT1 protein has a cell membrane location prediction. *PrSUT3* expression was low in tubercles but increased strongly in shoots during growth, concomitantly to a high hexose accumulation in vacuoles. Interestingly, PrSUT3 protein displays a tonoplast location prediction. We propose that PrSUT1 and PrSUT3 could play complementarily roles in parasitic sink areas for sucrose unloading from phloem and sucrose transport in vacuoles, respectively.

PS18-589

Endophytic *Bradyrhizobium* fix nitrogen in sweet potatoes

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Recently, nitrogen fixation by endophytic diazotrophs has been observed in a wide variety of plants. Sweet potatoes (*Ipomoea batatas*) are known for its ability to grow well in nitrogen-poor condition and the possible input of N by biological nitrogen fixation was suggested. To clarify the contribution of endophytic nitrogen fixation, we isolated and identified diazotrophic endophyte associated with sweet potatoes. The isolate, which possess *nifH* , a gene encoding one of the subunits of nitrogenase, were identified as strains of *Bradyrhizobium* sp. AT1 based on their 16S rRNA gene sequences. The *B*. sp. AT1 showed acetylene reduction activity in sweet potato extracts under micro-aerobic conditions. We also examined the infection of *B*. sp. AT1 in sweet potatoes and their influence on the growth and N_2-fixation as assessed by acetylene reduction method and ^{15}N dilution method. The inoculation of *B*. sp. AT1 resulted in an increase in the shoots and roots fresh weight compared to uninoculated control. The acetylene reduction activity was also detected in the stems of inoculated plants. Moreover, the ^{15}N atom% excess values of the shoots in inoculated sweet potatoes were lower than those in control plants. These results suggested that the isolated *B*. sp. AT1 expresses nitrogenase activity in sweet potatoes and contributes to their nitrogen nutrition.

PS18-590

Vertical and horizontal transmission of endophyte fungus *Neotyphodium lolii* in perennial ryegrass (*Lolium perenne* L.) plants

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The aim of our studies was to assess the extent to which endophytes move vertical in ecotypes of perennial ryegrass, and whether there are any signs of horizontal transmission of endophytes. Materials for vertical transmission analysis were ecotypes collected from permanent grasslands from three separate regions in Poland in a form of living plants. Plants, seed collected from this plants and plants grown from mentioned seed were tested for endophyte infection. The highest endophyte presence was noted in ecotypes from Swietokrzyskie region, while lower in Podlasie and Mazowsze regions. It was found that vertical transmission of *Neotyphodium* endophyte in perennial ryegrass ecotypes is almost complete, provided all produced seed will be viable and able to germinate and produce seedlings. For the experiment on horizontal transmission, four cultivars were selected. Endophyte-hosting plants (E+) and endophyte-free plants (E-) of mentioned varieties were planted the field in close distance on small plots and frequently mown. After 7 months plants grown in the middle and at the edge of each plot were examined for the presence of endophytes mycelium. The results indicated the possibility of horizontal transmission of endophytes between plants, especially between plants grown side by side. The studies have shown that in E- plants, after 7 month of growth next to the E+ plants, the characteristic mycelium of *Neotyphodium* spp. were found. This was especially true for plants growing in close distance to the inhabited plants. The possibility of above plant-to-plant transmission of endophyte has not been demonstrated so far.

PS18-591

Germination stimulants of *Phelipanche ramosa* in the rhizosphere of *Brassica napus* are derived from the glucosinolate pathway

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Phelipanche ramosa is a major parasitic weed of *Brassica napus*. The first step in host-parasite interaction is stimulation of parasite seed germination by compounds released from host roots. However, germination stimulants produced by *B. napus* have not been identified yet. In this study, we characterised the germination stimulants that accumulate in *B. napus* roots and are released into the rhizosphere. Eight glucosinolate-breakdown products were identified and quantified in *B. napus* roots by GC-MS. Two (3-phenylpropanenitrile and 2-phenylethyl isothiocyanate, 2-PEITC) were identified in the *B. napus* rhizosphere. Among these glucosinolate-breakdown products, *P. ramosa* germination was strongly and specifically triggered by isothiocyanates, indicating that 2-PEITC plays a key role in the *B. napus-P. ramosa* interaction. Known strigolactones were not detected by UPLC-MS/MS and seeds of *Phelipanche* and *Orobanche* species that respond to strigolactones, but not isothiocyanates, did not germinate in the rhizosphere of *B. napus*. Modification of strigolactone exudation by P starvation is known in strigolactone-producing plants. In contrast, P starvation did not affect *P. ramosa* seed germination in *B. napus* rhizosphere while S deficiency did, in concordance with the involvement of glucosinolate pathways in the synthesis of germination stimulants in *B. napus*. Furthermore, both wild-type and strigolactone biosynthesis mutants of *Arabidopsis thaliana* Atccd7 and Atccd8 induced similar levels of *P. ramosa seed* germination, suggesting that compounds other than strigolactone function as germination stimulants for *P. ramosa* in other *Brassicaceae*. Our results open perspectives on the high adaptation potential of root parasitic plants under host-driven selection pressures.

PS18-592

Transcriptome analysis of the parasitic plant *Phtheirospermum japonicum* indicatesrole of Subtilisin-like proteases in plant parasitism

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Parasitic plants invade host roots to take water and nutrients through aspecialized structure, the haustorium. To find the genes essential for parasitism the transcriptome of the facultative parasite

Phtheirospermum japonicum on autotrophic and parasitic stages were sequenced. The *de novo* assembly resulted in 58,137 contigs with minimum size of 300 bp. Among the transcript enriched from parasitic stage, many of the highly expressed sequences were annotated as subtilisin-like serine proteases (*SBT*). To investigate the role of these proteases in the plant parasitism, their expression patterns were monitored by qRT-PCR during the interaction of the parasite *Phtheirospermum* with a host(*Oryza sativa*) and a nonhost (*Lotus japonicus*) plants for 1, 2, 3 and 7 days of infection. The expression of three *SBT* genes (*PjSBT1*, *PjSBT2* and *PjSBT3*) was detected only at 7 days after the interaction with the host, while no expression of these genes was detected in contact with nonhost *L. japonicus*. In the similar way, the expression of genes encoding to subtilisin-like serine proteases from another parasitic plant *Striga hermonthica* was observed only when the parasite is infecting a susceptible host. Morphological studies of haustoria stained by Safranin-O revealed that the formation of vascular bridge which connects xylem vessels between a host and a parasite occurs after 7 days of interaction. Taking together, our results suggest a possible role of subtilisin-like serine proteases in parasitization.

PS18-593

Functional analysis of NADPH oxidases and regulatory factors of fungal endophyte *Epichloë festucae* in cell fusion and conidiation

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The endophytic fungus *Epichloë festucae* systemically colonize the intercellular spaces of host grass to establish a mutualistic symbiotic association. Colonization of epichloë endophytes confers various benefits on host plant growth and fitness to environmental conditions, including enhancement of plant tolerance to drought, disease and insect herbivory. Previously, we have shown that reactive oxygen species produced by a specific NADPH oxidase isoform, NoxA, and associated regulators NoxR and RacA have critical role in regulating hyphal growth in the host plant and establishment of symbiosis. We also have identified homolog of yeast polarity protein, BemA as an interactor of NoxR. In this study, we assessed morphological characteristics of mutants of Noxs and their regulators in culture. On nutrient rich medium, most of tested mutants grew normally but *racA* mutants showed hyphal swelling and increased branching. On poor medium, in contrast, *noxA*, *noxB* and *noxR* mutants showed increased hyphal branches and swelling, whereas *bemA* showed hyphal winding. While WT strains produced few conidia, *noxA* mutants showed significant increase of conidiation, and *noxAB* mutants produced further more conidia, suggesting that NoxA and NoxB have redundant functions to regulate conidiation. Unexpectedly, increased production of conidia was not observed in *noxR* and *racA* mutants. Although WT strains frequently formed hyphal fusions, most of tested mutants totally lost the ability to form hyphal fusions expect *bemA* mutant that showed significantly reduced number of hyphal fusions. Our study indicated that Noxs and these regulators have distinct or overlapping functions for the regulation of different hyphal morphogenesis.

PS18-594

Draft genome sequences of the parasitic *Striga* species

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Striga spp. (common name witchweeds) are parasitic plants belonging to Orobanchaceae. *Striga* infests major crops and causes devastating yield losses in sub-Saharan Africa and parts of Asia. To develop efficient methods controlling *Striga* infestation, the molecular basis of parasitism should be studied in detail. However, the molecular and genomic resources of Striga species have been limited. Among *Striga* spp., *Striga asiatica* is ideal material for complete genome sequence assembly because of its relatively small genome size (approx. 600 Mbp) and self-pollinating nature. We first cultured a single *S. asiatica* plant in axenic condition to obtain a large volume of homogeneous genomic DNA. Six paired-end and mate-pair libraries of different insert sizes were constructed and more than 200 Gbp sequences were obtained by the Illumina HiSeq2000 sequencer. These short reads were assembled and resulted in the scaffolds with N50 size of over 120 Kbp. About 50% of the genome is occupied by repeat sequences, suggesting *Striga* genome is expanded by repeat sequences like other plant species. The obtained genome sequence is being annotated using the information of closely related plant genomes and transcriptome sequences. In addition, a BAC library with 5-times coverage was prepared and its end-sequences will be analysed by the Sanger sequencer to improve the quality of the *S. asiatica* genome sequence assembly. *We acknowledge the sequence support by RIKEN GeNAS.

PS18-595

Identification of a novel fungal nuclear protein, NsiA, essential for symbiotic infection of endophytic fungus *Epichloë festucae*

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The endophytic fungus *Epichloë festucae* systemically colonize the intercellular spaces of host grass plant to establish a mutualistic symbiotic association. Previous studies have established that *E. festucae* and related endophytes confer bioprotective benefits to their host plants. A screen to identify symbiotic genes isolated a fungal mutant FR405 that altered the interaction from mutualistic to antagonistic. Perennial ryegrass infected with this mutant become severely stunted, show precocious senescence, as previously isolated *noxA* (a NADPH oxidase) mutant. FR405 has plasmid insertion in the coding region of uncharacterized Alanine Glycine Serine and Proline-rich protein. As GFP fusion of this protein was localized to nuclei, we designated this gene *NsiA* for nuclear protein for symbiotic infection. We have found that *noxA* mutant lost the ability to form hyphal fusions and showed significant increase of conidiation. *nsiA* mutant also showed no hyphal fusion as *noxA* mutant, however, conidiation was normal as wild type. These results indicated that NsiA is involved in similar processes to NoxA, but play roles downstream of NoxA in the signal transduction for the regulation of symbiotic hyphal growth. By yeast two-hybrid assay, it was shown than NsiA can directly interact with Ste12, a C_2/H_2-Zn^{+2} finger transcription factor, implied that NsiA regulate the activity of transcription factor to control symbiotic hyphal growth of endophyte in host plant.

PS19-596

Effects of sowing density on yield and quantitative characteristics of soybean

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In order to investigate the effects of different densities on yield and yield components in soybean, an experiment was conducted in a factorial based on randomized complete block design with three replications at research farm, Islamic Azad University of Kermanshah at 2007-2008. Cultivars factor were placed in the blocks at 3 levels including M7, M9, and Gorgan3 and density factors at 3 levels including plant were placed on 3, 5, 7 cm intra

rows spacing (53, 32 and 23 plant/m^2) in the blocks. The end of growth stage and harvesting time, the grain yield and yield components were determined. The results showed that density of 23 and 53 plant/m^2 had highest and lowest numbers of branches per plant, respectively. The highest number of node per plant and 100 grain weight per (main stem, branches and plant) related to M7 cultivar and highest number of pod per (branches and plant) related to Gorgan3 cultivar. also M7 and Gorgan3 had highest number of grain per plant and number of grain per branches, respectively. A significant correlation coefficient were found between grain yield with plant height (r=0.71**), number of grain per plant (r=0.73**), 100 grain weight (r=0.43**), biological yield (r=0.85**) and harvest index (r=0.34**). Gorgan3 had highest yield than two cultivars, M7 and M9. The highest yield related to density of 23 of plants/m^2.

PS19-597

Multiple mechanisms for soil phosphate solubilization: acid and more

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Phosphorus is an essential plant nutrient. In agriculture, soluble mineral phosphate fertilizer is used to increase productivity. Rock phosphate, from which this fertilizer is derived, is a dwindling non-renewable resource, which creates a future food security concern. In soil, soluble phosphate is readily trapped as insoluble phosphate complexes, typically with Al, Fe, or Ca. The phosphate solubilizing (PSOL) fungus, *Penicillium bilaiae*, in Novozymes' JumpStart liberates soil phosphate, reducing fertilizer use and attendant problems. PSOL fungi can acidify the soil surrounding them, liberating phosphate for plant growth. However, Al and Fe phosphate are soluble at alkaline pH, whereas Ca phosphate is soluble at acid pH. It appears that PSOL mechanism(s) may differ between P minerals. We examined three PSOL fungi with distinct lifestyles (soil dwelling, endophytic, rhizoplanar: *Penicillium bilaiae* and two novel isolates, respectively) for their ability to solubilize Al, Fe, and Ca phosphate in dilute potato dextrose broth. Each of these PSOL strains solubilized all of Al, Fe, and Ca phosphates, but used distinct mechanisms based on medium pH changes. Only *Penicillium bilaiae* strongly acidified the medium. Thus, PSOL activity is more complex mechanistically than previously thought.

PS19-598

Host-induced gene silencing in fungal pathogens of cereals

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RNAi is an established means to knock-down genes in plants and fungi. Both sequence-specificity as well as systemic spreading of gene silencing is essentially mediated by small interfering RNAs. Considering that there is intimate cellular contact between plants and fungal pathogens during infection, we hypothesized that fungal genes may effectively be targeted by RNAs derived from appropriately designed hairpin constructs expressed by host plant cells. This phenomenon called host-induced gene silencing (HIGS) has already been described in plant parasitic nematodes, insects, and parasitic plants. *Blumeria graminis* is a powdery mildew-causing fungus which infects many grass species including cereals. Intensive research is going on to better understand the barley-powdery mildew pathosystem, which is an established experimental model for biotrophic plant-pathogen interactions. Thirty nine independent transgenic barley lines were generated using a hairpin RNAi construct targeting a fragment of the *B. graminis hordei GLUCANOSYLTRANSFERASE 1* (*GTF1*) gene. GTFs are specifically found in fungi where they are involved in cell elongation and virulence. We have chosen GTF1 as a HIGS-target since it was found to be significantly upregulated during host colonization. In three of the T_1-populations obtained, colony formation of *B. graminis* was significantly reduced whereas a transgenic control line lacking the hairpin cassette was as susceptible as wild-type control plants. The results suggest uptake of RNA molecules by the powdery mildew fungus from attacked plant cells, which may cause knock-down of targeted fungal genes and reduced disease severity as a means to effectively combat fungal diseases of crop plants.

PS19-599

Effect of compost and chemical fertilizer on vegetable growth and microbial community structure in soil

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Compost provides a rich growing medium that useful to improve physical and chemical property of soil. Application of chemical fertilizer to soil is expected to improve plant growth by supplying one or more plant nutrients. The application of both compost and chemical fertilizer affects microbial structure and activity in soil. On the other hand, applying excessive amounts of chemical fertilizer has negative effects such as inhibit microbial growth that responsible for humus formation. To avoid over-application, it is important to study the optimum application of the fertilizer. In this research, the effect of compost and chemical fertilizer on vegetable growth of komatsuna (*Brassica rapa* var. *perviridis*) were investigated. The compost and chemical fertilizer were applied to soil simultaneously at different composition. Microbial community structure in soil was also characterized by microbial quinone profile. The results showed that at a mixture of 50% compost and 100% chemical fertilizer, the shoot length of 12.3 cm was the highest among all the applications. At a recommended application, 100% compost and 100% chemical fertilizer, the shoot length was 8.7 cm. There was also a change on microbial community structure after application of the compost and chemical fertilizer. Total quinone concentration of the best soil for the vegetable growth was 8.3 µmol/kg-dry soil in which menaquinone-7, menaquinone-6, menaquinone-8(H_2), menaquinone-10(H_4), menaquinone-10 were the most dominant among 12 microbial quinones species detected.

PS19-600

Engineering plant cell walls for second generation biofuel production

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The substrate for second generation biofuels is lignocellulosic material obtained from plant cell walls. Genetic modification of the cell wall has the potential to improve cellulose accessibility and hydrolysis, therefore decreasing the cost and energy input in biofuel production. This study aims to improve understanding of cell wall biosynthesis and organisation to increase cellulose content and extractability by genetic modification and pretreatment with white rot fungus *Phanerochaete chrysosporium*. Enzymatic saccharification assays have shown differences in soluble sugars released from transgenic tobacco lines down-regulated in both lignin and xylan. Significantly, *TOBACCO PEROXIDASE 60* down-regulated line 1074 shows 30% increase in glucose release as compared to the wildtype. Xylan down-regulation by suppression of *UDP-GLUCURONATE DECARBOXYLASE*, which synthesises the xylan precursor xylose, also caused improvement in saccharification. Treatment of the cell wall modified lines with *P. chrysosporium*, a white rot fungus that naturally hydrolyses

and metabolises lignin further improved saccharification after pretreatment. We also show that lignin biosynthesis pathway is down-regulated at the transcriptional level in lignin modified lines, while the polysaccharide biosynthesis response differs depending on the position of disruption in lignin biosynthesis.

PS19-601

Evaluation of IRES-mediated translation efficiency of viral 5' untranslated regions (UTRs) by multi-color luciferase reporter system in higher plants

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Internal Ribosome Entry Site (IRES) allows cap-independent translation initiation. Recent studies revealed the presence of IRES-mediated translation mechanisms among plant viruses. It has been shown that the 5' UTR sequence from several plant viruses can mediate cap-independent translation in plant cells. In order to evaluate various IRES-dependent translation efficiencies in plant cells, we have developed a dual-color bioluminescence reporter system using luciferase genes derived from a luminous click beetle, *Pyrophorus plagiophthalamus*. Two luciferase genes, designated CBR and CBG, are separated by an IRES element and expressed under the control of the *Cauliflower mosaic virus* 35S promoter. Although they share 99% identical amino acid sequences, the CBR emits red light while the CBG emits green light. These luciferases catalyze D-luciferin that is also catalyzed by firefly luciferase, and the red and green light can be separated by the optical filters. Using an appropriate detection system, we are able to monitor the IRES activity as a red/green ratio *in planta*. To further improve the assay system, we combined *Renilla* luciferase (hRluc) as internal standard for CBR and CBG. This makes it possible to evaluate the relative activity of CBR and CBG, respectively. Towards the understanding of the 5' UTR functions and the application for the multi-gene expression system in plant cells, we are currently using this technology for characterization and evaluation of IRES activities of 5' UTRs from various plant viruses.

PS19-602

Detection of quantitative trait loci for partial blast resistance by next-generation whole genome re-sequencing in the rice cultivar Nortai

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Rice blast, which is caused by *Magnaporthe oryzae*, is a highly destructive disease of rice widely distributed throughout the major rice growing regions of the world. The rice cultivars Nortai and Hitomebore show high and low levels of partial resistance to leaf blast, respectively. To detect the quantitative trait locus (QTL) conferring resistance in Nortai, we first developed a set of 241 recombinant inbreed lines (RIL) from the cross between Nortai and Hitomebore as a mapping population. The conventional QTL mapping involves linkage analysis using such RILs. However, this approach couldn't be applied in this study. Nortai and Hitomebore both belong to the *O. sativa* spp. *Japonica* sub-group, and are therefore genetically closely related. Although their closeness permits easy observation of the segregation in quantitative traits such as partial resistance, it poses a difficulty in developing polymorphic DNA markers for conventional linkage analysis. To overcome this problem, we developed a novel approach for QTL identification using next-generation whole genome re-sequencing of two bulked DNAs of progeny showing extreme phenotypic values. This approach allows the rapid identification of QTL because it does not require DNA marker development, the most time-consuming and costly procedure associated with the conventional QTL analysis. In the current study, we applied this new method for the detection QTLs conferring partial resistance in Nortai and detect the major QTL on chromosome 6. Additional experiments are underway to isolate the gene associated with this major QTL.

PS19-603

Development of endophytic bacterial inoculants possessing growth promotion traits for practical application in bio-energy plant species

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Internal plant tissue colonisation has made endophytic bacteria valuable for agriculture as a tool to improve crop performance particularly for those bacteria having traits such as plant growth promotion (PGP). This project involved screening and identifying endophytic bacterial strains, sourced from the bacterial collection available at the Institute of Technology Carlow, with the potential to enhance growth rate in bio-energy plant crop species. In the initial stage under greenhouse condition, a total of 140 strains in master mix (MM) groups of 10 strains, were inoculated into Rye Grass. The inoculated Rye Grass seeds were sown in pots and arranged in complete randomised design and their growth was monitored for a period of 3 months. The plant fresh weights (FW) and dry weights (DW) were used as growth parameters. Three MMs (comprising 30 strains) showed PGP potential in Rye Grass, significantly increased the mean FW and DW of Rye Grass plants compared to the negative controls. The 30 selected strains were further characterised for PGP traits under *in vitro* study. Results showed three strains inhibited *Pythium* spp. growth in dual culture assay, whereas the culture filtrates to quantify gluconic acid production necessary for inorganic phosphate solubilisation, had six strains recording more than 20mg/ml of gluconic acid production. Ten strains showed Indole acetic acid (IAA) production in the range (10-18 µg/ml) while three strains showed 1-aminocylopropane-1-carboxylic acid (ACC) deaminase activity. This study indicates that the selected bacterial endophytes have the potential for PGP and development in plant crops.

PS19-604

Homology-independent breakdown of papaya transgenic resistance by super virus strain and the solution

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Papaya ringspot potyvirus (PRSV) limits the production of papaya worldwide. Underlying the mechanism of post-transcriptional gene silencing (PTGS), we generated CP-transgenic papaya lines with resistance to different strains of PRSV. However, during field trials, an unrelated *Papaya leaf-distortion mosaic potyvirus* (PLDMV) able to break down the CP-transgenic resistance was noticed. To overcome this threat, transgenic papaya lines carrying a chimeric untranslatable construct with partial CP coding sequences of both PRSV and PLDMV were further generated to confer double resistance to both viruses. However, during field tests of double virus-resistant lines, super strains of PRSV able to overcome the single-virus or double-virus resistance were discovered. The recombination between PRSV super strain 519 and common strain YK indicated that a recombinant virus containing the silencing suppressor HC-Pro from the super strain 519 can break down

the transgenic resistance in a transgene-homology independent manner. Agroinfiltration of PRSV YK and PRSV 5-19 gene silencing suppressor HC-Pros further confirmed that 5-19 HC-Pro has stronger capability of gene silencing suppression. Comparison of YK and 5-19 HC-Pros revealed variations in five aa positions in the region responsible for gene silencing suppression and genome amplification. Consequently, new transgenic papaya lines carrying an untranslatable HC-Pro construct of the supper PRSV strain 519 to disarm its ability of suppressing PTGS were generated. These transgenic lines conferred complete resistance to PRSV super strains and other PRSV geographic strains. Currently, we are pyramiding single, double, and super transgenic resistances in a papaya hybrid variety for global application.

PS19-605

Development of abaca (*Musa textilis* Nee) putative resistant lines against *Banana bunchy top virus* and *Banana bract mosaic virus* through induced mutation breeding

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Abaca (*Musa textilis* Nee), or known worldwide as Manila hemp, were *in vitro* propagated and subjected to varying dosages of gamma irradiation Cobalt 60 (^{60}Co). Irradiated shoots were sub-cultured (M_1V_4-M_1V_5) to minimize chimeras and evaluated for radiosensitivity response and post-radiation recovery. LD_{30} was established at 10-15 Gy on cvs. Tinawagan Pula (TP) and Tangongon (TG) with high shoot proliferation and plant vigor 45 days after irradiation. Two stages of screen house inoculation tests were conducted on both irradiated and non-irradiated control plants. *Banana bunchy top virus* (BBTV) and *Banana bract mosaic virus* (BBrMV) were inoculated using aphids and mechanical transmission, respectively. Screening tests showed 0.6% TP and 0.9% TG putative resistant (PR) lines were consistently found negative to BBTV and 1.6% TP and 0.9% TG uninfected with BBrMV. Consequently, field trial showed that propagated PR lines (mother plant) were 92.75% (64/69) consistently uninfected with BBTV whereas 66.67% (46/69) were found negative to BBrMV and 59.4% (41/69) were free from both viruses. In addition, detection assays also confirmed the complete absence of endo- and exogenous badnavirus in abaca in which commonly observed from micropropagated banana. As gamma irradiation dose coupled with *in vitro* propagation was established in this study, screening for and generating PR lines can be fast-tracked over the conventional breeding works with safe mutation against badnavirus.

PS19-606

A single chain variable fragment antibody against a *Fusarium virguliforme* toxin for enhancing tolerance of soybean to sudden death syndrome

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Plants do not produce antibodies. However, plants can correctly assemble functional antibody molecules encoded by mammalian antibody genes. Many plant diseases are caused by pathogen toxins. One of such diseases is the soybean sudden death syndrome (SDS). SDS is a serious disease caused by the fungal pathogen, *Fusarium virguliforme*. The pathogen, however, has never been isolated from the diseased foliar tissues. Thus, one or more toxins produced by the pathogen have been considered to cause foliar SDS. One of these possible toxins, FvTox1, was recently identified. We investigated if expression of anti-FvTox1 single chain variable fragment antibody in transgenic soybean can confer resistance to foliar SDS. We have created two single-chain variable fragment (scFv) antibody genes, *Anti-FvTox1-1* and *Anti-FvTox1-2*, encoding anti-FvTox1 scFv antibodies from RNAs of a hybridoma cell line that expresses mouse monoclonal anti-FvTox1 7E8 antibody. Both anti-FvTox1 scFv antibodies interacted with an antigenic site of FvTox1 that binds to mouse monoclonal anti-FvTox1 7E8 antibody. Binding of FvTox1 by the anti-FvTox1 scFv antibodies, expressed in either *Escherichia coli* or transgenic soybean roots, was initially verified on nitrocellulose membranes. Expression of Anti-FvTox1-1 in stable transgenic soybean plants resulted in enhanced foliar SDS resistance as compared to that in non-transgenic control plants. Our results suggest that (i) FvTox1 is an important pathogenicity factor for foliar SDS development, and (ii) expression of scFv antibodies against pathogen toxins could be a suitable biotechnology approach for protecting crop plants from toxin-induced diseases.

PS20-607

Deep-sequencing of multiple race-leaf rust, *Puccinia triticina* genomes and transcriptomes during wheat infection

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The initial generation of *P. triticina* genomic resources (reference plus three isolate genomes [1] www.broadinstitute.org and ESTs [2]) spawned follow-up projects targeted at revealing genome fluidity among historical field isolates. Many isolates resulted from rust races that overcame introductions of resistant wheat varieties The investigations aim to reveal alteration in fungal gene expression during wheat infection with the goal of identifying the molecular basis of fungal adaptation to various host cultivars. Thatcher and near-isogenic wheat lines carrying resistance gene *Lr2a* or *Lr3* were inoculated with *P. triticina* isolates of virulence phenotype BBBD, MBDS, SBDG or FBDJ (producing a variety of infection types, from IT0 to IT4). Total RNA was isolated from infected leaves at various time points during the infection. In addition, 30 isolates were inoculated on susceptible cv Thatcher and total RNA was isolated from infected leaves 5 dai. Deep-sequencing using the Illumina platform was performed to assess transcriptomes. Fungal and wheat tags were separated via bioinformatics. All fungal genomes were also sequenced to support transcripts, and to reveal variation (SNPs) and uniqueness among isolates. A major focus is on effectors. However, comparative analyses to poplar leaf rust, *Melampsora larici-populina* fungal isolate transcriptomes is also performed to analyze commonality and variation between these rusts. Funding: Strategic Opportunities Fund3, Genome British Columbia (R Hamelin, G Bakkeren); Ontario Research Fund - Research Excellence (B Saville, B McCallum, G Bakkeren); [1] Inter-agency NSF/USDA-CSREES Microbial Genome Sequencing Program (C Cuomo, J Fellers, L Szabo, G Bakkeren). [2] Xu et al., 2012. BMC Genomics 12: 161.

PS20-608

An analysis of gene families evolution in Sordariomycetes reveals a high level of gene loss in many phytopathogens

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The class Sordariomycetes includes species with many different life styles. Among them we can found human pathogens, entomopathogens, fungi pathogens and many important

phytopathogens, including those from genera *Fusarium*, *Gibberella*, *Verticillium*, *Ceratocystis* and *Magnaporthe*. The class also include saprotrophs, some from extreme habitats and holding enzymes which have been recently studied for biotechnological purposes. Additionally, two of the most important model species among fungi - *Neurospora crassa* and *Podospora anserina* are Sordariomycetes. Therefore, it is not a surprise that this class presents a large number of complete genome sequences publicly available, making the Sordariomycetes one of the most suitable fungi groups to perform comparative genomics studies. In this work we investigate the patterns of gene gain and loss in 16 Sordariomycetes, including saprotrophs, plant pathogens and fungi pathogens, as well the models species *N. crassa* and *P. anserina*. The results indicate a remarkable heterogeneity in the rates of gene gain and loss among different species. Moreover, we detect strong bias favoring gene loss in many phytopathogens, including *Cryphonectria parasitica*, *Magnaporthe grisea* and especially, *Ceratocystis cacaofunesta*. A bias in favor to gene loss was also found in the genus *Neurospora*, but at a smaller rate. We hypothesize that the tendency to gene loss could be related to a generalized occurrence of repeat-induced point (RIP) mutation in these pathogens, since RIP is described in many Sordariomycetes.

PS20-609

Various stress conditions induce somatic homologous recombination in *Magnaporthe oryzae*

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The rice blast fungus *Magnaporthe oryzae* is widely recognized as one of the most variable pathogens and many races have been identified. New pathogen races can emerge through multiple gene modification mechanisms, such as modifying or removing the *Avirulence* (*Avr*) gene from their genome. In this study, we focused on one type of gene modification mechanisms, in *M. oryzae*, namely upon somatic homologous recombination (HR). In order to detect HR and calculate its frequency, we designed a recombination substrate, two nonfunctional recipient and donor genes from an *enhanced yellow fluorescent protein* (*EYFP*) / *blasticidine S deaminase* (*BSD*) fusion gene that allowed visual detection of the HR events as the restoration of EYFP fluorescence and BS tolerance. The constructed substrate genes were transformed into *M. oryzae* (isolate Hoku-1) and the effect of various stress conditions were examined using the spores obtained from the transformants. Chemical Stress conditions increased the frequency of HR, especially primary metabolism inhibitors. In addition, HR was induced by methyl viologen treatment. Methyl viologen causes an overproduction of reactive oxygen species within *M. oryzae* cells. Moreover, one of the first plant responses to microbial attack is to produce extracellular reactive oxygen species around the infection sites. Thus, we inoculated the spores to susceptible rice cultivar (Nihonbare) and reisolated spores from typical blast lesions. Reisolated spores showed higher frequency of HR than original spores. These results suggest that the increased genetic flexibility might facilitate evolutionary adaptation of *M. oryzae* to chemical stress and plant resistance.

PS20-610

Genomic and transcriptomic analysis of two *Colletotrichum* species

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Members of the genus *Colletotrichum* represent a group of hemibiotrophic plant pathogens, infecting a wide-range of commercially important crops. With the aim of developing a better understanding of the molecular basis of diseases caused by this genus, the genomes of *Colletotrichum orbiculare*, the causal agent of cucurbit anthracnose, and *Colletotrichum gloeosporioides*, isolated from strawberry, were sequenced and analysed. This revealed the expansion of the *C. orbiculare* genome, but no corresponding increase in gene content. Candidate effectors from both genomes were identified and it was found that the majority of small, secreted proteins in gene families were conserved between species. Transcriptomic analysis of *C. orbiculare* during pathogenesis of *N. benthamiana* was performed providing insight into genes and processes important for pathogen establishment and maintenance of infection.

PS20-611

Genomics, transcriptomics and proteomics analyses of the fungal pathogen *Ceratocystis cacaofunesta* provide new insights on the control of the wilt disease of cacao

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Ceratocystis cacaofunesta is an ascomycete (Class Sordariomycetes) that causes a lethal disease in cacao, leading to great economic losses in the infected areas. The development of control strategies relies on the knowledge of the pathogen biology and on the understanding of the mechanisms involved in its interaction with the host. In this context, integrated *-omics* approaches have proven their potential to provide valuable information of fungal-plant interactions. This work presents the analysis of the *C. cacaofunesta* genome (30.4 Mbp with 126 X coverage). Gene prediction showed the existence of 7.200 genes, which were validated by RNA-seq. We observed a drastic reduction in the number of gene families in comparison to other Sordariomycetes. This work describes the more contracted gene families and discusses the possible implication of this phenomenon with the fungus lifestyle. Our results indicate that *C. cacaofunesta* presents repeat-induced point mutation (RIP) for genome defense, explaining the low density of genes. Furthermore, using genomics, transcriptomics and proteomics approaches, we present a comprehensive analysis of the mitochondrial function in *C. cacaofunesta*. The fungal mitochondrion appears to contain a total of 1480 putative proteins, of which 23.4% (347) was identified by LC-MS/MS. Remarkably, three unknown proteins and a group of 35 hypothetical polypeptides are among the experimentally validated proteins. We studied the effects of mitochondrial targeted inhibitors on fungal growth. Our results suggest a possible participation of the alternative respiratory pathway in the *C. cacaofunesta* development and open new possibilities to control diseases caused by Ceratocystis species.

PS20-612

Difference in pathogenicity relating genes of *Magnaporhe oryzae* infecting ryegrass and rice based on next-generation genome sequencing

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Ryegrass blast by *Magnaporhe oryzae* is a major disease in temperate regions of Japan and caused by the same fungal species with rice blast. But it never attacks rice and a host specific pathogenicity is differentiated in ryegrass blast. The structure of genomes of ryegrass blast fungus is known to differ from rice blast one based on rep-PCR analysis, even if they are the same species. We analyzed the genome of the ryegrass blast fungus by a next-generation sequencer and compared the pathogenicity relating genes with those of rice blast fungus. In results of 84000 reads on sequences, 10692 contigs (29Mb) were obtained and it was thought to cover about two third of the whole genome. Among the pathogenicity relating genes detected, polyketide synthetase gene, protein kinase C, ubiquitin activating enzyme gene, cyclic AMP depending protein kinase and so on had 100% similarity compared with those of rice blast fungus on nucleotide bases. But triacylglycerol lipase gene, thiamine-pyroric acid synthetic enzyme gene, membrane penetrating protein subunit gene, GTPase Rab gene that acts as molecular switches on signaling and so on relating to cell membrane signaling, had 98.8-99.1% similarity. The genes that differ from those of rice blast fungus are assumed to contribute to the specific pathogenicity against ryegrass and the possibility will be discussed.

Poster 20

PS20-613

Comparative genome structure analysis and screening of pathogenicity related genes of *Magnaporthe* isolates by SOLiD whole genome resequencing

Shingo Urushizaki[1], Yuhko Kobayashi[1], Issei Kobayashi[1]
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Magnaporthe oryzae is a causal agent of blast disease on gramineous plants. Different isolates of the fungus from various host plants show different host specificity. Signal perception between the plant and the fungus appear to occur by no later than penetration attempts of the fungus, 18-24h after inoculation. However, molecular basis of nonhost recognition in the early infection stage still remains to be elucidated. To identify fungal effectors, which play important role in the early signal perception, genome sequence of six *Magnaporthe* isolates from rice, common millet, foxtail millet, finger millet and crabgrass were re-sequenced by the next generation sequencer, SOLiD and genome structures were compared with reference genome of a *M. oryzae* rice isolate, 70-15. Between rice isolates, KEN53-33 and 84-10B, comparing with 70-15, percentages of genes containing SNPs were considerably low, whereas much more SNPs containing genes were found in genomes of common millet and foxtail millet isolates. On the other hand, there are markedly large number of such genes in a finger millet and a crabgrass isolates. These results are suitable to known molecular phylogenic relationships, in which finger millet and the crabgrass isolates are far-related to other isolates. Moreover, 42 genes were screened as candidate of rice isolates specific effector genes, which are commonly preserved in rice isolates and absent in other isolates. These candidate genes contained some well-known effecter genes, such as *BAS1*. The possibility is suggested that some of these genes play important roles in determination of host specificity of *M. oryzae*.

PS20-614

Structure of a conditionally dispensable pathogenicity chromosome of the tomato pathotype of *Alternaria alternata*

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The tomato pathotype of *A. alternata*, the causal agent of the stem canker of tomato, produces host-specific AAL-toxin and induces apoptotic cell death on susceptible tomato cells. AAL-toxin is structurally similar to a mycotoxin fumonisin produced by unrelated fungi *Gibberella* (*Fusarium*) spp. AAL-toxin biosynthetic (*ALT*) gene cluster consists of at least 13 genes homologous to the fumonisin biosynthetic (*FUM*) genes in *F. verticillioides* has been identified in the tomato pathotype. The *ALT* cluster includes genes for polyketide synthase (*ALT1*), cytochrome P450 monooxygenase (*ALT2*) and others. Functional analysis of the *ALT* genes showed that some of them are involved in AAL-toxin biosynthesis by the pathogen and pathogenicity/virulence against susceptible tomatoes. The *ALT* cluster locates only on the 1.0 Mb small chromosome found in the tomato pathotype. Based on biological and pathological observations, the small chromosome was indicated to be a conditionally dispensable chromosome (CDC) and a pathogenicity chromosome. We have proposed a hypothesis for the ability to produce AAL-toxin and to infect plant could potentially be distributed among *A. alternata* by horizontal transfer of the CDC. The complete sequence of the CDC of the tomato pathotype has been determined using 454 FLX sequencing. The sequence data led to identification of many novel ORFs in addition to the *ALT* genes, such as genes for transporters, transcription factors and cell signaling factors. The *ALT* cluster is located on the subtelomeric regions of the CDC and transposase-like sequences were found around the cluster.

PS20-615

The *LaeA*-like methyltransferase gene (*AaLAE*) regulates host-specific toxins production and pathogenicity in the fungal plant pathogen *Alternaria alternata*

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Filamentous fungi produce a variety of secondary metabolites. The LaeA gene, a global regulator of secondary metabolites, has been identified in *Aspergillus nidulans*. *LaeA* encodes methyltransferase and regulates production of secondary metabolites such as penicillin and aflatoxin in *Aspergillus* spp. In this study, we identified *LaeA* ortholog (*AaLAE1*) that encodes a methyltransferase by whole-genome draft sequencing of the tomato pathotype of *Alternaria alternata* known to produce a toxic secondary metabolite, host-specific AAL-toxin, as a disease determinant on susceptible tomatoes. AAL-toxin production and pathogenicity of *AaLAE1*-deleted mutants were significantly reduced and spore production and hyphal growth were also affected. Gene expression of the AAL-toxin biosynthetic gene cluster was reduced in the mutant. *LaeA* homologues were further identified in the strawberry and apple pathotypes of *A. alternata* and designated *AaLAE2* and *AaLAE3*, respectively. Production of host-specific AF- and AM-toxins was reduced in the *AaLAE2* and *AaLAE3* mutant, respectively, with a decrease of virulence on each host plant. The mutants also showed decreased aerial hyphal growth and sporulation. Thus, *AaLAE* genes positively regulate host-specific toxins biosynthesis, pathogenicity and hyphal growth of the *A. alternata* pathotypes.

PS20-616

Genetic change of *Pyricularia grisea* in different host genome

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Grass has been known as an alternative host beside rice for the rice blast fungus. The aim of this study was to investigate the ability of

genetic change of blast fungus of grass around the rice field when they passed through different host genome. Assessment of genetic change during host alteration was investigated through the genetic analysis of the basal samples of isolated *Pyricularia* from *Digitaria ciliaris* after infection this isolate into rice and *Panicum repens* using SCAR markers (Cut1, Erg2, PWL2), rep-Pot2, Amplified fragment length polymorphism (AFLP) and pathotype. The result of this study showed that cross infection of *Pyricularia* d4 from *D. ciliaris* grass into rice induced genetic variation in their Cut1 and PWL2 markers, AFLP and rep-Pot2 patterns, as well as pathotype. The infection into susceptible rice plant could induced genetic change more higher than into resistant plant. However, the infection into different genus of grass (*P. repens*) induced genetic change only in AFLP and rep-Pot2 patterns, but not in SCAR markers. Result of this study indicated that the cross infection in different host genome might induce microevolution of *Pyricularia* d4.

PS20-617

Evolutional origin of the conditionally dispensable chromosomes controlling pathogenicity of *Alternaria alternata* pathogens

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The filamentous fungus *Alternaria alternata* includes seven pathogenic variants (pathotypes), which produce host-specific toxins (HST) and cause necrotic diseases in different plants. The HST biosynthetic genes have been isolated from six pathotypes (Japanese pear, strawberry, tangerine, apple, tomato and rough lemon) of *A. alternata*. The genes for biosynthesis of each toxin are located at the same locus in the genome, defining a gene cluster. The HST biosynthetic gene clusters of the six pathotypes were found to reside on single small chromosomes of <2.0 Mb in most strains tested. Loss of the small chromosomes was observed in the strawberry, apple and tomato pathotypes, and the small chromosomes appeared to be conditionally dispensable (CD) chromosomes. We determined the structures of the CD chromosomes of the strawberry, apple and tomato pathotypes and identified putative toxin biosynthetic gene cluster regions on the chromosomes. Pairwise comparison of the entire regions of CD chromosomes from these pathotypes identified large syntenic regions among the three CD chromosomes. The regions including HST gene clusters are unique to the respective pathotypes, but the remaining regions of CD chromosomes from the three pathotypes are conserved. The co-linear order of genes has been maintained within the CD chromosomes of the three pathotypes. These results suggest that the CD chromosomes have a common origin, and that the syntenic regions are the core of the original, dispensable chromosome.

PS20-618

Genome evolution of fungal pathogens from *Magnaporthe oryzae*/*grisea* clade

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Magnaporthe oryzae is a fungal species complex gathering pathogens of different Poaceaes that causes the main fungal disease of rice and severe epidemics on wheat in South America. This project aims at characterizing genomic determinants and evolutionary events involved in the adaptation of fungi to different host plants. Eight strains from *M. oryzae* species complex pathogenic on either rice, wheat, *Setaria* or *Eleusine* and one strain of the related species *M. grisea* pathogenic on *Digitaria*, have been sequenced using NGS. De novo annotation was carried out with Eugene for gene and with REPET for transposons. Most frequent families are LTR retro-transposons, but some DNA transposons were found. Repeats cover about 10-12% of these genomes. Variable genome sizes (36-42 Mb) and gene contents (12 300-20 500 genes) were estimated for these genomes, even though 4 genomes were more fragmented (poor scaffolding, short and truncated CDS). OrthoMCL analysis including *M. oryzae* 70-15 reference genome, identified 20 443 clusters, including 8 154 single copy shared by-all families (core genome) and variable number of species-specific gene families (305-1550). Gene families expected to be involved in pathogenicity including genes encoding enzymes involved in the biosynthesis of secondary metabolites, enzymes involved in plant cell wall degradation and small secreted peptides are currently analyzed. 12-14% of the predicted CDS encode putative secreted proteins with a median length of 260 aa. A dedicated database was developed to facilitate evolutionary analyses and integration of RNAseq data from in planta infection kinetics. Additional comparative analyses will be presented.

PS21-619

Structural insights into TIR domain and effector function in effector-triggered immunity in flax and Arabidopsis

Bostjan Kobe[1], Thomas Ve[1], Simon Williams[1,2], Li Wan[1], Maud Bernoux[3], Pradeep Sornaraj[2], Emma de Courcy-Ireland[2], Jeffrey G. Ellis[3], Peter A. Anderson[2], Peter N. Dodds[3]
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Effector-triggered plant immunity is initiated through the recognition of a pathogen effector protein by a plant resistance (R) protein, leading to the activation of plant defenses and a localized cell death response. The effectors usually have roles in virulence and are structurally diverse, while R proteins generally fall into a few conserved families. We have used the fungal pathogen flax rust interaction with flax as a model system to characterize this process. The flax R proteins consist of a core nucleotide-binding domain, an N-terminal Toll-interleukin 1-receptor (TIR) domain, and a C-terminal leucine-rich repeat (LRR) domain. We have shown the direct interaction of the effector proteins AvrL567 and AvrM with R proteins L6 and M, respectively, and also determined the crystal structures of AvrL567 and AvrM. Recently, we determined the crystal structure of a TIR domain from L6 at 2.3 A resolution. The structure reveals important differences from the structures of mammalian TIR domains, and highlights three separate functionally important protein surfaces, involved in oligomerization, interaction with a downstream signaling partner, and regulatory intramolecular interactions, respectively. We have also complemented this work with a study of the TIR domains of Arabidopsis proteins RPS4 and RRS1, which work in concert to confer resistance to several pathogens. Our results bring us a step closer to understanding the molecular basis for the disease resistance process.

PS21-620

Structure analysis of *Tomato spotted wilt virus* nucleocapsid proteins

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Tomato spotted wilt virus (TSWV) is a member of *Tospovirus* genus in the family *Bunyaviridae*. TSWV infects over 650 plant species including many crops. TSWV virions are spherical membrane-bound particles decorated with two glycoproteins (Gn, Gc). The virions contain three negative/ambisense genomic RNAs (S, M, L), which are associated with nucleocapsid proteins (NP) and RNA polymerases (LP). NP plays various and significant roles in the life cycle of the virus. The NP-LP-genomic RNA complex in virions possesses an ability to synthesize RNA genomes. Interaction between NP and the viral glycoproteins is proposed to be required for the virion formation. However, the structure of NP of the genus Tospovirus has not been solved, and the interaction manner with other viral factors remains to be elucidated. We obtained crystals of recombinant TSWV NPs diffracting to 2.7 Å resolution. The structure was solved by SAD method using a Se-Met derivative. The asymmetric unit contains three NP molecules forming trimeric ring-like structure. Each of the N- and C terminal domains of NP forms an arm-like structure, which interacts with adjacent NP. Moreover, the inside of TSWV NP trimeric ring shows the positive charge rich surface, suggesting that these regions are expected to be genomic RNA binding site.

PS21-621

Crystal structure of a flax cytokinin oxidase and interaction studies with a fungal effector

Li Wan[1], Markus Koeck[2], Simon Williams[1], Xiaoxiao Zhang[1], Jeffrey Ellis[2], Peter Dodds[2], Bostjan Kobe[1]
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The flax rust effector AvrL567-A has been shown to physically interact with the flax resistance protein L6. This interaction activates the L6 protein, which results in the initiation of the hypersensitive response defense pathway. Using yeast-2-hybrid assays and bimolecular fluorescence complementation assays in planta, we have identified another host protein, the cytokinin oxidase LuCKX1, to interact with AvrL567-A. LuCKX1 is closely related to AtCKX7, one of seven proteins in *Arabidopsis thaliana* responsible for irreversible degradation of cytokinins. These plant hormones are involved in developmental processes such as lateral root formation and their cellular concentration changes during pathogen infection. Currently, the function of AvrL567-A during flax rust infection is unknown and we are interested in determining if the targeting of LuCKX1 promotes pathogen virulence. Interestingly, kinetic analysis has shown that the cytokinin oxidase activity of LuCKX1 increases in the presence of AvrL567-A. We determined the crystal structure of LuCKX1 at a resolution of 1.8 angstrom. Utilizing the structure of LuCKX1 and the previously determined AvrL567-A structure, we are investigating the interaction interface between the two proteins. We anticipate that this combined structural and biochemical study will provide insight as to the function of AvrL567-A during infection and the role of LuCKX1 in disease resistance and/or susceptibility.

PS21-622

The GTP-form structure of small GTPase OsRac1, a key player in rice innate immunity

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Plants have a number of defense mechanisms to protect them from infection by pathogens. In *Oryza sativa* (rice), OsRac1, a plant small GTPase and a member of Rac/Rop family, has emerged as a key activator of downstream defense processes upon elicitor mediated signaling. For example, a constitutively activated mutant of OsRac1 shows increased resistance to rice bacterial blight disease, due to an increased formation of reactive oxygen species (ROS) and subsequent cell death. On the contrary, a dominant-negative mutant of OsRac1 shows decreased resistance. These results suggest that OsRac1 plays an important role as a molecular switch in plant innate immunity. However, the molecular mechanisms by which OsRac1 is activated in innate immunity remain largely unknown. Here, we have determined a crystal structure of OsRac1 at resolution of 1.9 Å complexed with a non-hydrolyzable GTP analog GMPPNP, as a first step for better understanding the molecular mechanisms of defense in plants. This is the first GTP-form structure of the plant small G protein.

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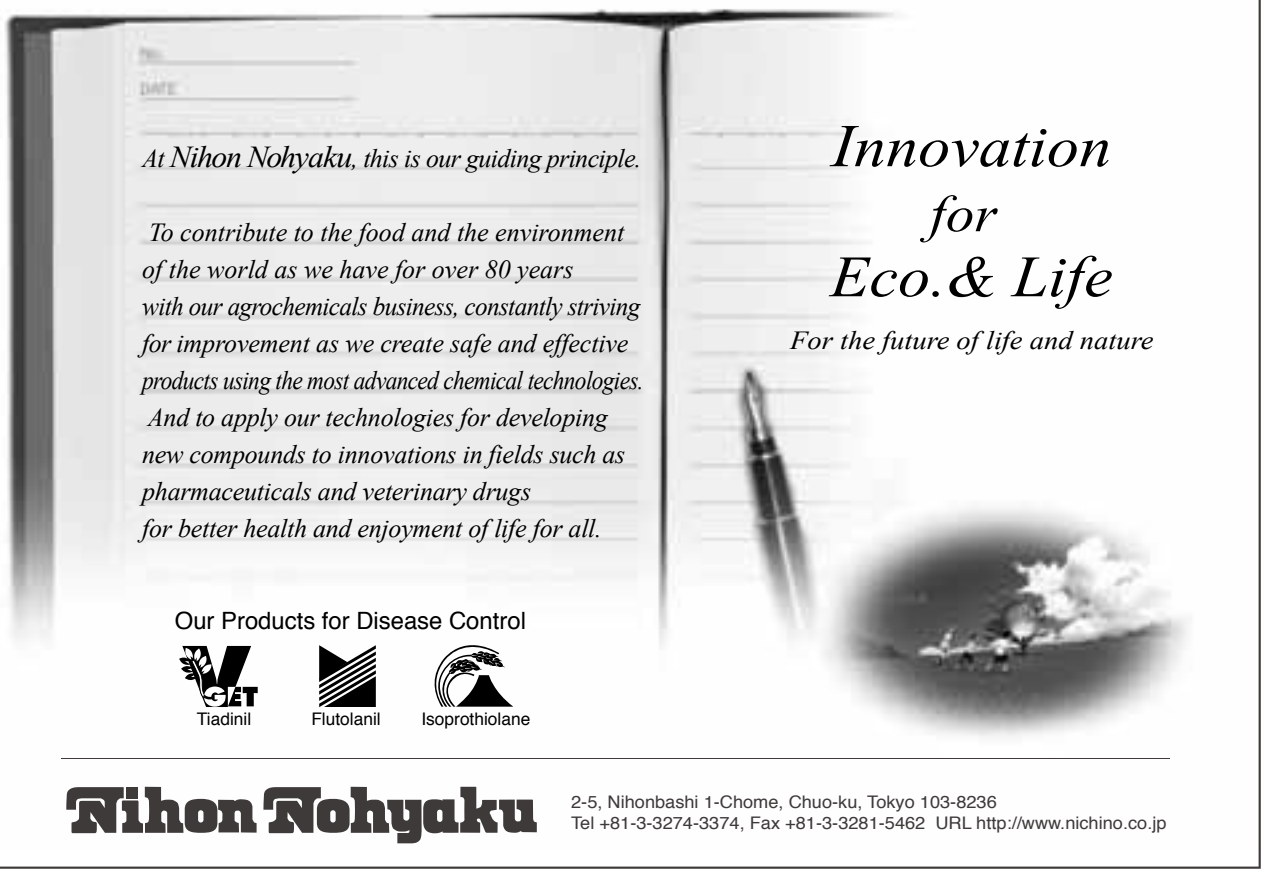

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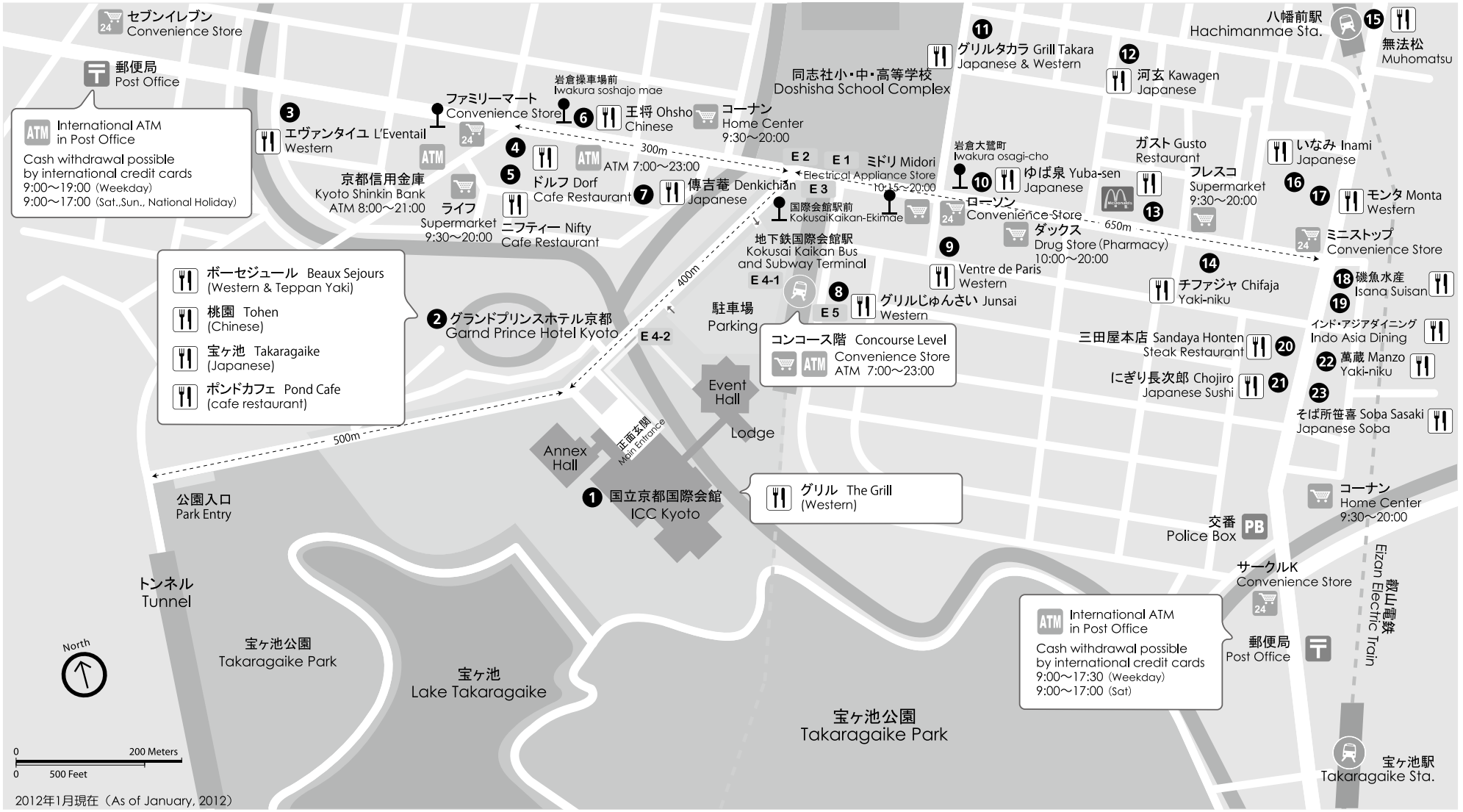

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グリル タカラ Grill Takara	075-722-7461	10:30〜20:00 At random dates	Japanese & Western L D ¥800〜	11
いなみ Inami	075-721-5796	11:30〜14:30 (14:00) 17:30〜21:00 Tue., 2nd & 4th Wed.	Pork cutlet P L D ¥1,000〜	16
磯魚水産 Isana suisan	075-708-6752	11:30〜14:30 (weekend) 17:00〜22:30 Mon.	Japanese Seafood L ¥1,000〜 D ¥2,000〜	18
河玄 Kawagen	075-721-6813	17:00〜21:00 Thu.	Japanese Kappo D ¥8,000〜	12
無法松 Muhomatsu	075-711-2744	17:30〜01:00 At random dates	Japanese Izakaya D ¥2,000〜	5
そば処 笹喜 Soba Sasaki	075-712-7879	11:30〜15:00 17:00〜20:00 Wed. (except holiday)	Soba P L D ¥1,000〜	23
ゆば泉 Yuba-sen	075-703-2700 takaragaike.yubasen.co.jp	11:00〜15:00 17:00〜21:00 No holiday	Bean Curd Restaurant P L D ¥2,500〜	10

WESTERN｜洋食

Restaurant	Telephone / URL	Open / Closed	Style	MAP
ドルフ Dorf	075-722-2367	09:00〜22:30 Jan.1st	Cafe Restaurant P L D ¥1,000〜	4
エヴァンタイユ L'Eventail	075-712-0750 www.eventail.jp	11:30〜13:30 17:30〜20:30 Mon.	French P L D ¥5,000〜	3
ガスト Gusto	075-705-2035 www.skylark.co.jp	09:00〜05:00 No holiday	Restaurant P L D ¥500〜	13
グリルじゅんさい Junsai	075-721-1035 kyoto-junsai.com	11:30〜14:00 17:00〜21:00 Wed. (except holiday)	Western P L D ¥1,000〜	8
モンタ Monta	075-722-1711	11:30〜14:00 18:00〜21:00 Wed.	Western P L ¥1,000〜 D ¥2,000〜	17
ニフティー Nifty	075-722-2370	11:00〜22:30 Jan.1st	Cafe Restaurant P L D ¥1,000〜	5
三田屋本店 Sandaya Honten	075-702-1129 sandaya-honten.co.jp	11:30〜14:30 17:00〜21:30 Year-end and new-year	Steak House P L ¥2,000〜 D ¥3,000〜	20
ヴァントレ・ドゥ・パリ Ventre de Paris	075-722-0999	11:30〜14:00 17:30〜20:00 Thu. (except holiday)	French P L ¥1,000〜 D ¥2,000〜	9

ASIAN｜アジア料理

Restaurant	Telephone / URL	Open / Closed	Style	MAP
チファジャ Chifaja	075-791-1129 www.inami.jp	17:00〜01:00 No holiday	Yakiniku (Roast meat) P D ¥2,000〜	13
インド・アジア・ダイニング Indo Asia Dining	075-702-6911 indoasiadining.com	11:00〜15:00 17:00〜22:00 No holiday	Ethnic food L ¥1,000〜 D ¥2,000〜	19
萬蔵 Manzo	075-723-5556 manzo-kyoto.jp	11:00〜23:00 No holiday	Yakiniku (Roast meat) P L ¥800〜 D ¥2,000〜	22
王将 Ohsho	075-721-9183 www.ohsho.co.jp	11:00〜02:00 11:00〜22:00 (Sunday) No holiday	Chinese P L D ¥300〜	6

L Lunch　D Dinner　P Parking

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Hankyu railway

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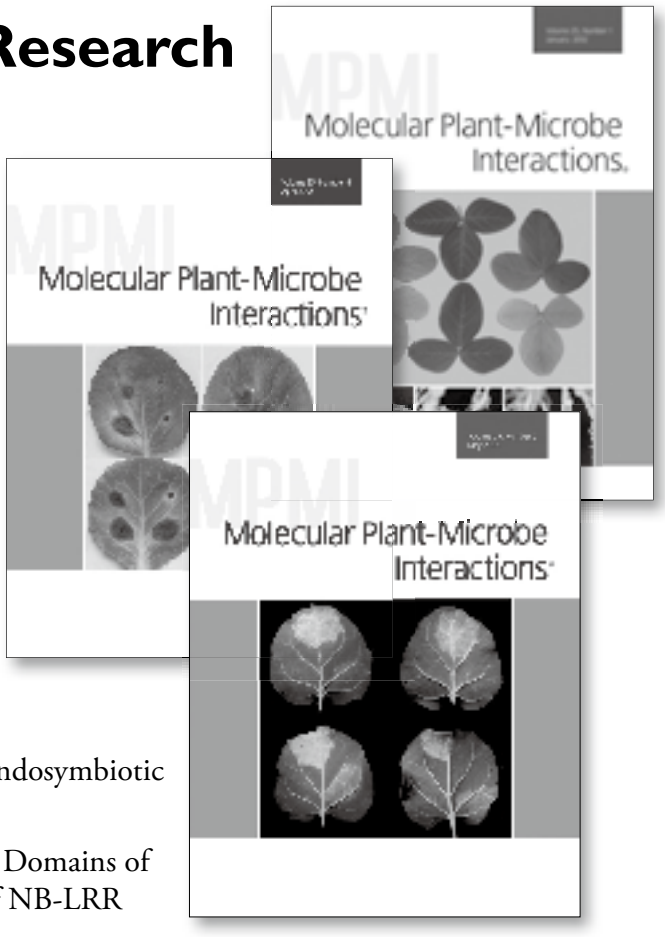

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- Lipo-chitooligosaccharide Signaling in Endosymbiotic Plant-Microbe Interactions
- Cell Death Mediated by the N-Terminal Domains of a Unique and Highly Conserved Class of NB-LRR Protein
- The Type VI Secretion System: A Multipurpose Delivery System with a Phage-Like Machinery
- Nonhost Resistance of Rice to Rust Pathogens
- *Medicago truncatula IPD3* Is a Member of the Common Symbiotic Signaling Pathway Required for Rhizobial and Mycorrhizal Symbioses
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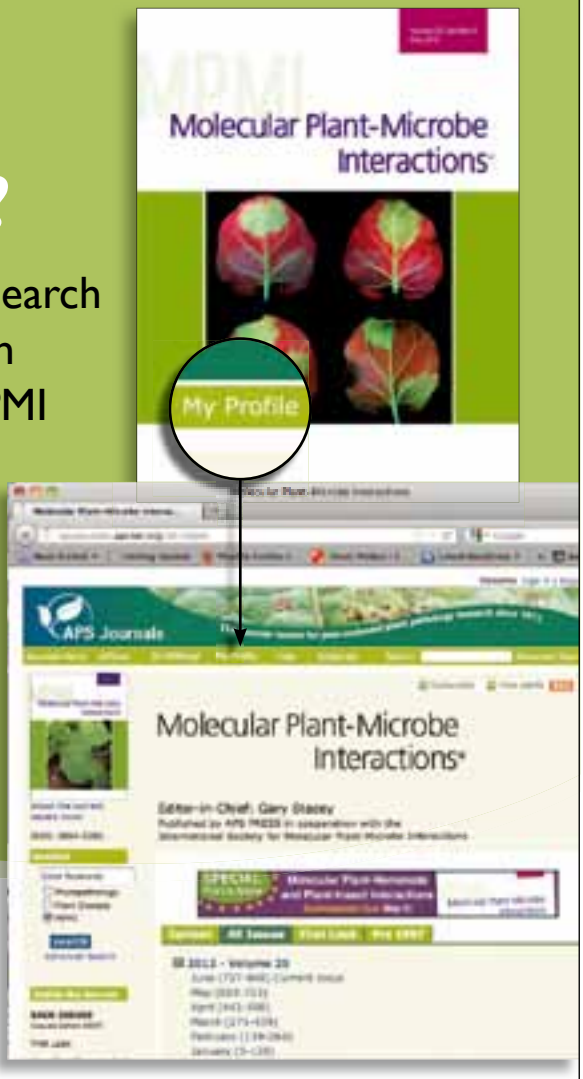

Jillian Lang added molecular diagnostics, *Xanthomonas oryzae*, and *Pythium ultimum* to her saved-searches and is notified when new content is published in ***MPMI Online*** that relates to these terms.

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