

Identification of pea lines resistant to Aphanomyces euteiches and related root architecture traits

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PROGRAM & ABSTRACT BOOK



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Sunday, July 6, 2014

15:00 – 18:30	Registration Open
	Open Forum: Science in Grain Legumes (Coordinated by Noel Ellis, CGIAR)
19:00 – 21:00	Welcome and Networking Reception for IFLRC VI & ICLGG VII delegates and Canadian Special Crops Association Convention (CSCA) delegates

Monday, July 7, 2014

Fundamental and Applied Genetics and Genomics

Location: Salons A, B, C (Upper Level)

7:30	Registration Open		
7:30– 8:45	Breakfast (Grand Salon - Upper I	_evel)	
9:00– 10:30	Plenary Session IFLRC VI & IC Co-chairs: Doug Cook (University of Califorr Tom Warkentin (University of Sas	nia Davis, USA)	
9:00– 9:45		Nevin D. Young (University of Minnesota, USA) Exploring Nodulation through Genome Resequencing and Association Genetics in Medicago	
9:45– 10:30		Judith Burstin (INRA, Dijon, FRANCE) Recent pea genomic resources will enhance complementary improvement strategies in this crop	
10:30- 11:00	Refreshment Break		
11:00- 12:30	Concurrent Sessions		
ICLGG Fundamental Ge Chair: Doug Cook (L	Gallery A, B (Main Level) enomics Iniversity of California Davis, USA)	IFLRC Gallery C, D (Main Level) Applied Genetics & Genomics Chair: Tom Warkentin (University of Saskatchewan, Canada)	

11:00-11:30 Scott Jackson (Univ. of Georgia, USA) "Genetic and epigenetic variation in legumes: focus on <i>Glycine max</i> and <i>Phaseolus vulgaris</i> "	11:00-11:30 Noel Ellis (CGIAR, Hyderabad, India) Shifting the focus: populations, genetics and genomics
11:30-11:50 Stig Andersen (Aarhus University, Denmark) <i>Lotus japonicus</i> natural variation and LORE1 insertion mutants	11:30-11:45 Rachit K. Saxena (ICRISAT, India) Whole genome re-sequencing based classification of heterotic pools for accelerating hybrid breeding in pigeonpea
11:50-12:10 Greg Perry (University of Guelph, Canada) Using SNP genotyping to identify regions of <i>Phaseolus</i> <i>acutifolius</i> introgression in OAC-Rex, a CBB resistant variety of <i>Phaseolus vulgaris</i>	11:45-12:00 Ping Wan (Beijing University of Agriculture) A high-density SNP genetic map and QTL mapping for yield and other agronomic traits in adzuki bean (<i>Vigna angularis</i>)
12:10-12:30 Stephan Schröder (North Dakota State University, USA) Optimization of Genotyping by Sequencing (GBS) Data in Common Bean (<i>Phaseolus vulgaris</i> L.)	12:00-12:15 Trupti Joshi (University of Missouri, Columbia, USA) Next Generation Resequencing of Soybean Germplasm for Trait Discovery
	12:15-12:30 Gilles Boutet (INRA, Rennes, France) SNP discovery in pea: A powerful tool for academic research and breeding

12:30 - 14:00

Lunch Break (Grand Salon - Upper Level)

14:00 - 15:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level) Evolution Chair: Sachiko Isobe (Kazusa DNA Research Institute, Japan)	IFLRCGallery C, D (Main Level)Applied Genetics & Genomics; DiseaseResistanceChair: N.P. Singh (IIPR, Kanpur, India)
14:00-14:30 Peter Tiffin (University of Minnesota, USA) Targets of selection in the <i>Medicago</i> genome	14:00-14:15 Janila Pasupuleti (ICRISAT, India) Identification of introgression lines with superior pod yield and improved rust resistance in peanut (<i>Arachis hypogaea</i> L.)
14:30-15:00 Maren Friesen (Michigan State University, USA) Symbiotic Dimensions of Trifolium Biodiversity	14:15-14:30 Ehsan Sari (University of Saskatchewan) Genotype-dependent interaction of lentil lines with <i>Ascochyta</i> <i>lentis</i>
15:00-15:20 Steven Cannon (USDA-ARS) Multiple incidents of polyploidy and nodule evolution near the origin of the legumes	14:30-14:45 Judith Lichtenzveig (Curtin University, Australia) Pathogenomics of the <i>Didymella</i> spp. causal agents of Ascochyta blights in legumes
15:20-15:40 Elisa Bellucci (Università Politecnica delle Marche, Ancona, Italy) Decreased nucleotide and expression diversity and modified co-expression patterns characterize domestication in the common bean	14:45-15:00 Jagmeet Kaur (Punjab Agricultural University, Ludhiana, India) Moisture stress induced changes in metabolites and cellular functions of chickpea (<i>Cicer arietinum</i> L.) genotypes
	15:00-15:15 Renu Singh (CCS Haryana Agricultural University, India) Mapping micronutrients using recombinant inbred lines (RILs) in Mung bean (<i>Vigna radiata</i> L.)

15:15-15:30 **Larn McMurray** (SARDI, Adelaide, Australia) Improved weed control in Australian pulse production through advancements in genetic and agronomic research

15:40 - 16:00 Refreshment Break

16:00 – 18:00 Concurrent Sessions

ICLGG Gallery A, B (Main Level) Development Chair: Sachiko Isobe (Kazusa DNA Research Institute, Japan)	IFLRCGallery C, D (Main Level)Applied Genetics and Genomics; Abiotic StressToleranceChair: Ashutosh Sarker (ICARDA, India)
16:00-16:30 Catherine Rameau (INRA, Versailles , France) Strigolactones and other long distances signals regulating shoot branching	16:00-16:30 Steve Beebe (CIAT, Cali, Colombia) Breeding for tolerance to low soil phosphorus: the example of common bean
16:30-17:00 Jim Weller (University of Tasmania, Australia) Genetic control of flowering in temperate legumes	16:30-16:45 Shane Rothwell (Lancaster University, UK) Liming (to recommended rates) limits legume growth and gas exchange by increasing root-to-shoot signalling of the phytohormone abscisic acid
17:00-17:20 Jianxin Ma (Purdue University, USA) Molecular Basis of Soybean Stem Architecture	16:45-17:00 Ralf Metzner (Institute of Plant Sciences, Jülich, Germany) Monitoring roots, nodule and pod development in vivo: New perspectives on legume development
17:20-17:40 Julie Hofer (ICRISAT, Patancheru, India) Sequence analysis of a pea (<i>Pisum sativum</i>) fast neutron mutant population identifies the gene corresponding to <i>Stipules reduced</i>	17:00-17:15 Jens Berger (CSIRO Plant Industry) Chickpea phenology and evolution
17:40-18:00 Jocelyn Ozga (University of Alberta, Canada) Role of gibberellins in seed coat development and photoassimilate partitioning in developing pea (<i>Pisum</i> <i>sativum</i> L.) seeds	17:15-17:30 Shiv Kumar (ICARDA) Extra short duration lentils for rice-based cropping systems in the Indo-Gangetic Plains of South Asia
	17:30-17:45 Imran Malik (University of Western Australia) Variation in physiological responses in pea and lentil to soil waterlogging
	17:45-18:00 Hari D. Upadhyaya (ICRISAT, India) Capturing Variations for Multiple Stress and Agronomic Traits for Improvement of Grain Legumes

18:30 - 22:00

Evening Event, Wanuskewin Heritage Park

Tuesday, July 8, 2014

Seeds and Nutrition

Location: Salons A, B, C (Upper Level)

7:30– 8:25	Breakfast (Grand Salon - Upper I	_evel)
8:30– 10:30	Plenary Session IFLRC VI & IC Co-Chairs: Georgina Hernandez (Universida Albert Vandenberg (University of	d Nacional Autónoma de México, Mexico)
8:30– 9:15	Murad Al-Katib (President and C Changing Face of Global Ag	CEO of Alliance Grain Traders Inc.) priculture
9:15– 10:00	Richard Thompson (INRA, Dijor Use of translational genomics	n, France) to identify genes important for legume seed development
10:00– 10:45		aylor College of Medicine, Houston, Texas, USA) od Crops: Recent Conceptual Developments and Strategic
10:45– 11:00	Refreshment Break	
11:00 - 12:30	Concurrent Sessions	
ICLGG Seeds and Nutrition Chair: Georgina Hernandez Autónoma de México, Mexi 11:00-11:30 Jerome Ver Biological Sciences, CAS, O Deciphering regulation of le composition	co) r dier (Shanghai Institutes of China)	IFLRC Gallery C, D (Main Level) Seeds and Nutrition Chair: Albert Vandenberg (University of Saskatchewan, Canada) 11:00-11:30 Francesca Sparvoli (IBBA, CNR, Milan, Italy) Iron biofortification in common bean Ital
Norwich, UK) The wild side of seed qualit	oney (John Innes Centre, y improvement: discovering impacts on the control of visual sum sativum L. (pea)	11:30-11:45 William Erskine (University of Western Australia) Selenium biofortification of lentil in Australia and Bangladesh
11:50-12:10 Mitch Lucas Riverside, USA) Using SNPs to Breed Cowp		11:45-12:00 Peter Zahradka (Canadian Centre for Agri-Food Research in Health and Medicine) The structural properties of arteries are altered by consumption of pulses: Implications for treatment of atherosclerotic disease
Biotic Stress 12:10-12:30 Vijai Bhadau Saskatchewan, Canada) Regulation of hemibiotrophy pathogen <i>Glomerella trun</i>	y in the lentil anthracnose	12:00-12:15 Laura McBreairty (University of Saskatchewan) A pulse-based diet and exercise training in women with polycystic ovarian syndrome: Effects on body composition, blood lipids and reproductive measures
		12:15-12:30 Susan Arntfield (University of Manitoba,

	Canada) Changes in levels of antioxidant and selected antinutritional factors in peas and beans due to the application of heat
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12:30 - 14:00 Lunch Break (Grand Salon - Upper Level)

12:45 - 14:00 Legume data integration meeting (Coordinated by Steven Cannon) – open forum

14:00 – 15:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level)	IFLRC Gallery C, D (Main Level)
Biotic Stress Chair: Scott Jackson (Univ. of Georgia, USA)	Seeds and Nutrition Chair: Khalid Daoui (Centre Régional de la Recherche Agronomique de Mèknes, Morocco)
14:00-14:30 Kiran Mysore (The Samuel Roberts Noble Foundation, USA) Identification of novel sources of resistance in a nonhost plant, <i>Medicago truncatula</i> , against Asian Soybean Rust caused by <i>Phakopsora pachyrhizi</i>	14:00-14:30 C.L. Laxmipathi Gowda (ICRISAT, India) Impact-Oriented Legume Seed Systems in developing countries of Sub-Saharan Africa and Asia
14:30-15:00 Nadine IIk (The Sainsbury Laboratory, Norwich, UK) Translational research at The Sainsbury Laboratory – from the laboratory to the farm	14:30-14:45 Felix Dapare Dakora (Tshwane University of Technology, South Africa) Role of symbiotic cowpea (<i>Vigna unguiculata L. Walp.</i>) as a major food legume for nutritional security in Africa
15:00-15:20 Marie-Laure Pilet-Nayel (INRA, Rennes, France) Translational genomics for resistance to <i>Aphanomyces</i> <i>euteiches</i> between <i>Medicago truncatula</i> and pea	14:45-15:00 Ramakrishnan Nair (AVRDC – The World Vegetable Center South Asia, India) Enhancing production and consumption of mungbean
15:20-15:40 Zenglu Li (University of Georgia, USA) An Integrated Molecular Breeding Approach for Breeding Nematode Resistance in Soybean	15:00-15:15 Jenny Wood (NSW Department of Primary Industries, Australia) Imaging of grain legume seeds: understanding what is where and the influence on nutritive value, health benefits and quality traits
	15:15-15:30 Arun Shunmugam (University of Saskatchewan) Biochemical and molecular characterization of low phytate pea lines

- 15:40 16:00 Refreshment Break
- 16:00 18:00 Poster Session (Odd number)
- 8:30 22:00 Banquet Reception, **Delta Bessborough Gardens**

Wednesday, July 9, 2014

Nitrogen Fixation, Plant Nutrition and Legume Mega Projects

7:30– 8:45	Breakfast (Grand Salon - Upper Level)
9:00– 10:30	Plenary Session IFLRC VI & ICLGG VII Co-Chairs: Jens Stougaard (Aarhus University, Denmark) C.L. Laxmipathi Gowda (ICRISAT, India)
9:00– 9:45	Kirstin Bett (University of Saskatchewan, Canada) Lentil genome sequencing: establishing a comprehensive platform for molecular breeding
9:45– 10:30	Ken Giller (Plant Production Systems, Wageningen University, The Netherlands) N2Africa: Putting nitrogen fixation to work for smallholder farmers in Africa
10:30– 11:00	Refreshment Break

11:00- 12:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level)	IFLRC Gallery C, D (Main Level)
Legume Mega Projects	N Fixation and Plant Nutrition
Chair: Jens Stougaard (Aarhus University, Denmark)	Chair: C.L. Laxmipathi Gowda (ICRISAT, India)
11:00-11:30 Tim Close (University of California Riverside, USA) Innovation Lab on Climate Resilient Cowpea	11:00-11:30 Endalkachew Wolde-meskel (Hawassa University, Ethiopia) Exploiting indigenous rhizobial biodiversity resources and symbiotic N2-fixation to benefit small-holder farmers: the case of Ethiopia
11:30-12:00 Doug Cook (University of California Davis, USA) Taking a walk on the wild side: prospecting for climate resilience and nitrogen fixation traits in the wild progenitors of cultivated chickpea	Ethiopia 11:30-11:45 Matthew Denton (University of Adelaide, Australia) A national survey of farmers in Australia to understand rhizobial inoculant use for pulse and pasture legumes
12:00-12:30 Suk-Ha Lee (Seoul National University, Korea) Genome Sequence of Mungbean and Vigna Speciation	11:45-12:00 Adriana Navarro-Borrell (Agriculture and Agri- Food Canada, Swift Current) Managing soil microbial resources and wheat yield through crop rotation in 4-year systems in the Canadian Prairie
	12:00-12: 15 Fran Walley (University of Saskatchewan) Assessment of Arbuscular Mycorrhizal Fungal Inoculants for Pulse Production Systems
	12:15-12:30 Navid Bazghaleh (University of Saskatchewan) Genotypic variation in the response of chickpea to arbuscular mycorrhiza and fungal endophytes

12:30 - 13:15

Lunch Break (Grand Salon - Upper Level)

	Breeding Field Tour Hutterite Colony ian Light Source
13:15 – 18:30 Weed	Cooking Class with Local Chef Research Plots at Scott e "Fun Run or Fun Walk"

Free evening

Thursday, July 10, 2014

Location: Salons A, B, C (Upper Level)

Biotic Stress and Plant Microbe Interactions

7:30– 8:45	Breakfast (Grand Salon - Upper Level)
9:00- 10:30	Plenary Session IFLRC VI & ICLGG VII Co-Chairs: Kirstin Bett (University of Saskatchewan, Canada) Phil Davies (SARDI, Adelaide, Australia)
9:00– 9:45	Jens Stougaard (Department of Molecular Biology, Aarhus University, Denmark) Bacterial infection of epidermal cells is controlled by a two-step recognition process
9:45– 10:30	Alain Baranger (INRA, Rennes, France) Plant architecture and development to control aerial disease epidemics in legumes: the case of Ascochyta blight in pea
10:30– 11:00	Refreshment Break

11:00 - 12:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level) Symbiosis Chair: Kirstin Bett (University of Saskatchewan, Canada)	IFLRC Gallery C, D (Main Level) Disease and Pest Resistance Chair: Carlota Vaz Patto (Universidade Nova de Lisboa, Portugal)
11:00-11:30 Pascal Ratet (Institut des Sciences du végétal CNRS, Gif sur Yvette, France) Identification of <i>Medicago truncatula</i> genes preventing development of plant defenses during symbiosis	11:00-11:30Weidong Chen (USDA/ARS, Washington State University, USA)What roles do fungal secondary metabolites play in interactions between Ascochyta fungi and cool season food legumes?
11:30-11:50 Georgina Hernandez (Universidad Nacional Autónoma de México, Mexico) MicroRNAs from common bean (<i>Phaseolus vulgaris</i>) in the rhizobia symbiosis and in the response to abiotic stress	11:30-11:45 Aurore Desgroux (INRA, Rennes, France) Identification of pea lines resistant to <i>Aphanomyces euteiches</i> and related root architecture traits
11:50-12:10 Jeremy Murray (John Innes Centre, Norwich, UK)	11:45-12:00 Beybin Bucak (Harran University, Sanliurfa, Turkey)

The Rhizobial Infectome: Uncovering the Genes that Control the Early Steps of the Legume-Rhizobia Interaction	Transfer of resistance to broomrape (<i>Orobanche crenata</i>) from <i>Lens ervoides</i> to cultivated lentil.
12:10-12:30 Hongyan Zhu (University of Kentucky, USA) Genetic control of symbiosis specificity in the legume- rhizobial mutualism	12:00-12:15 Kristy Hobson (NSW Department of Primary Industries, Australia) Developing superior chickpea varieties by improving chickpea Phytophthora resistance using wild relatives
	12:15-12:30 Diego Rubiales (Institute for Sustainable Agriculture, Cordoba, Spain) Exploitation of wild relatives in pea breeding for disease and pest resistance

12:30 - 14:00

Lunch Break (Grand Salon - Upper Level)

14:00 – 15:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level)	IFLRC Gallery C, D (Main Level)
Symbiosis	Disease and Pest Resistance
Chair: Jeremy Murray (John Innes Centre, Norwich, UK)	Chair: Sabine Banniza (University of Saskatchewan, Canada)
14:00-14:30 Krzysztof Szczyglowski (Agriculture and	14:00-14:30 Hari Sharma (ICRISAT, India)
Agri-Food Canada, London, Ontario, Canada)	Potential of host plant resistance to insects for pest
Turning on the nitrogen tap: how legumes keep the nutrient flowing	management in grain legumes: Progress and limitations
14:30-15:00 Masayoshi Kawaguchi (National Institute for Basic Biology, Okazaki, Japan)	14:30-14:45 Mustapha El-Bouhssini (ICARDA, Rabat, Morocco)
Two kinds of mobile signals mediate long-distance control	Progress in host plant resistance in chickpea to Leaf miner,
of nodulation	Liriomyza cicerina (Rondani)
15:00-15:30 Christine Lelandais-Brière (Université Paris Diderot- France)	14:45-15:00 Lars Kamphuis (CSIRO Plant Industry, Wembley, Australia)
MicroRNA networks involved in root architecture and environmental interactions in the model legume <i>Medicago</i> <i>truncatula</i>	Resistance to aphids in the model legume Medicago truncatula
	15:00-15:15 Kiran Sharma (ICRISAT, India)
	Transgenic pigeonpea for resistance to legume pod borer:
	moving towards product development
	15:15-15:30 Ashutosh Sarker (ICARDA, Beirut, Lebanon)
	Adaptation of lentil and grasspea under various cropping systems in South Asia

- 15:30 16:00 Refreshment Break
- 16:00 18:00 Poster Session (Even number)
- 16:00 17:30
 Mini symposium: Root Diseases in Legumes (Coordinated by Clare Coyne)

 Gallery C, D (Main Level)
- 18:30 21:00 Evening Event, Western Development Museum

Friday, July 11, 2014

Location: Salons A, B, C (Upper Level)

Abiotic Stress and Crop Management

7:30– 8:45	Breakfast (Grand Salon - Upper Level)
9:00- 10:30	Plenary Session IFLRC VI & ICLGG VII Co-Chairs: Noel Ellis (CGIAR, Hyderabad, India) Felix Dapare Dakora (Tshwane University of Technology, South Africa)
9:00– 9:45	Rajeev Varshney (CEG, ICRISAT, India) The 1000 Pulse Genome Sequencing Initiative: Connecting Genes to Traits
9:45– 10:30	Kadambot Siddique (University of Western Australia) Abiotic Stress in Cool Season Grain Legumes: Genetic and Agronomic Approaches
10:30- 11:00	Refreshment Break

11:00- 12:30 Concurrent Sessions

ICLGG Gallery A, B (Main Level) Applied Genomics	IFLRC Gallery C, D (Main Level) Abiotic stress
Chair: Noel Ellis (CGIAR, Hyderabad, India)	Chair: Kadambot Siddique (University of Western Australia)
11:00-11:30 Maria Monteros (The Samuel Roberts Noble Foundation, USA) Opportunities for Genomics-Assisted Breeding Approaches in Alfalfa (<i>Medicago sativa</i> L.)	11:00-11:30 Pooran Gaur (ICRISAT, India) High temperature tolerance in grain legumes
11:30-11:50 Sachiko Isobe (Kazusa DNA Research Institute, Japan) <i>In silico</i> SNP and SSRP discovery in horsegram genome sequences	11:30-11:45 Phil Davies (SARDI, Adelaide, Australia) Selecting pulses tolerant to temperature extremes: frost and heat stress tolerance in field pea and faba bean
11:50-12:10 Sujan Mamidi (North Dakota State University, USA) Genome-wide association studies to identify major QTL responsible for Iron Deficiency chlorosis in soybean (<i>Glycine max</i>)	11:45-12:00 Rosalind Bueckert (University of Saskatchewan) Cool leaf traits to reduce heat stress in pea
12:10-12:30 Hamid Khazaei (University of Helsinki, Finland) Synteny-based mapping of morphological, agronomic and quality traits in faba bean (<i>Vicia faba</i> L.)	12:00-12:15 Maryse Bourgault (University of Melbourne, Australia) Field Pea growth and yield response to elevated CO2 in the Australian Grains Free Air CO2 enrichment facility (AGFACE)
	12:15-12:30 Janine Croser (University of Western Australia) Recombinant inbred line populations in less than a year? A novel system for combining accelerated breeding with screening for abiotic stress in grain legumes.

12:30 – 12:45 Closing remarks

12:45 - 14:00 Lunch (Grand Salon - Upper Level)

Keynote Speaker: Fundamental and Applied Genetics and Genomics (ICLGG)



Nevin Young is a Distinguished McKnight University Professor at the University of Minnesota with joint appointments in the Department of Plant Pathology and the Department of Plant Biology. Previously, Dr. Young was director of the Plant Molecular Genetics Institute (PMGI) at Minnesota. Dr. Young received his B.A. at Indiana University in 1977 and Ph.D. at Yale University in 1984, followed by a NSF Postdoctoral Fellowship at Cornell University. He is author or co-author of more than 270 scientific publications, including 105 refereed publications and 18 book chapters with an overall h-index score of 57. His research highlights include the first mapping of a plant disease resistance gene using DNA markers, the first comparative genetic mapping of plant developmental genes, and the first bioinformatic analysis of plant disease resistance gene families. In 2003, Dr. Young became principal investigator on the NSF initiative to sequence the genome of the model legume, *Medicago truncatula*, which was published in *Nature* magazine in 2011.

Exploring Nodulation through Genome Resequencing and Association Genetics in Medicago

Young, N.D.1*. 1University of Minnesota. *(neviny@umn.edu)

Genome resequencing enables the discovery of candidate loci and provides insights into the genomic architecture of complex traits. The Medicago Hapmap Consortium (University of Minnesota, National Center for Genome Resources, Boyce Thompson, J. Craig Venter Institute, Hamline University) is resequencing the genome of Medicago truncatula to explore the genomics of nodulation. Previously, we resequenced 320 diverse accessions of M. truncatula and related taxa to discover >6,000,000 single nucleotide polymorphisms (SNPs). Subsequent genome-wide association studies (GWAS) of nodulation uncovered previously reported genes (eq, CaML3, NFP, SERK2) plus novel candidates associated with DNA repair, ubiquination, molecular chaperones and other nodule-upregulated loci of unknown function. Functional genomics experiments are now underway to validate these novel candidates. A subset of 30 lines from the GWAS panel are now being deeply sequenced and assembled independently from the published A17 reference. This approach is essential for the discovery of DNA elements not found in the reference sequence as well as for high confidence prediction of structural variants (SVs). Our resequencing work has already produced a reference quality assembly for R108, a *M. truncatula* genotype widely used in functional genomics. These independent *M. truncatula* genome assemblies also lay a foundation for creating a *Medicago* "pan-genome". Multiple lines of evidence indicate that dynamic gene families. including NBS-LRRs and nodule-specific cysteine-rich peptides (NCRs), play key roles in nodulation. Genome resequencing is beginning to reveal the evolution and architecture of these important gene families.

Keynote Speaker: Fundamental and Applied Genetics and Genomics (IFLRC)



Dr. Judith BURSTIN is Director of research at INRA UMR1347 Agroecology Dijon-F. With an initial background in agronomy, quantitative genetics and breeding, she has acquired competencies in genomics and post-genomics. Her research focuses on pea and aims at deciphering the control of seed yield and quality in the context of climate change. A major scope of her research is the molecular understanding of plant adaptation. She leads a group working on the genetics and genomics of protein crops (mainly pea and fababean). She has been coordinating large multi-disciplinary projects in the field of pea quantitative molecular genetics, translational genomics and proteomics, and of development of molecular tools and plant material. She co-authored more than 30 papers in peer-reviewed international journals and 5 book chapters. She is expert for the French variety registration office and has been vice-president of the former European association of grain legumes.

Recent pea genomic resources will enhance complementary improvement strategies in this crop

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Pea has lagged behind many other crops in terms of dedicated genomic tools for several reasons including its large genome size (ca. 4.5 Gb). The recent development of high-throughput sequencing and genotyping technologies has permitted pea genomics to catch up and enter into the genomic era. We will present the development of functional and structural genomic resources in pea and their application in pea breeding. We developed a pea Unigene expression atlas that allows visualization of expression profiles for any gene of interest in various tissues subjected to different conditions. This was the basis for generating a high density microarray for functional genomics and a high-throughput genotyping BeadChip array for genotyping. These tools will be extremely useful in three breeding strategies followed by the team: (i) ideotype breeding, which pursue an ideal plant model that is expected to meet the desired yield and quality requirements of producers and users; (ii) targeted breeding, which chase the good combinations of genetic factors controlling traits of interest; and (iii) genome-wide breeding, which models phenotypes from high density marker genotypes.

Keynote Speaker



Murad Al-Katib, as President and CEO of Alliance Grain Traders Inc., is the guiding vision in all aspects of the business, also serving on the Board of Directors. After completing a Masters from Thunderbird School of Global Management in Arizona and a Bachelor of Commerce from the University of Saskatchewan, Murad worked in international trade promotion for the Government of Saskatchewan before founding Saskcan Pulse Trading in 2001, providing the nucleus for Alliance Grain Traders. Over the next decade, the company has grown to become a world leader in value-added pulses, staple foods and ingredients selling to over 100 countries around the globe and with 29 manufacturing plants in 5 continents.Murad has served on the Board of Directors of the Canadian Special Crops Association and Pulse Canada and served as Chair of the Advisory Board for Small and Medium Enterprise for the Canadian Minister of International Trade. Murad also served as a member of the Panel for the Renewal of Canada's Global Commerce Strategy. Murad is also the recipient of a number of prestigious awards including the 2012 Pulse Promoter Award from BASF and Saskatchewan Pulse Growers, the 2004 Ernst and Young Emerging Entrepreneur of the Year and one of 2005's Canada's "Top 40 under 40" by the Caldwell Partners and the Globe and Mail.

Changing Face of Global Agriculture

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As the world population rises, global demand for protein is rising in emerging markets and consumer tastes and preferences in non-traditional markets for pulses such as North America and Europe are leading to robust new demand. The discussion will examine these trends and explore the role of innovation, product development and R&D in meeting the new opportunities ahead in the global pulses industry. The speaker will use AGT as a case study in discussing regional development of the global pulses industry and discuss current and future trends that will drive the consumption of pulses.



Richard D. Thompson. After a Ph.D. in Molecular Biology U. Edinburgh (1979) and periods at the PBI Cambridge UK, and the MPI for Plant Breeding Research, Köln, currently researcher in the INRA Agroecology Unit, Dijon, (2002-present, Grain Legumes Unit Director 2002-2010), focussing on the genetic control of seed development and composition in legumes, using the *Medicago truncatula* model, and more recently, Pea (*Pisum sativum*). By applying proteomics and transcriptomics approaches, gene expression profiles for isolated tissues of the seed have been obtained and clusters of co-regulated genes acting during discrete stages in seed filling identified. To narrow down candidate genes for the control of seed composition and yield, we have selected genes that co-locate with QTLs for these traits and are analysed the phenotypes of corresponding TILLING and TnT1 insertion mutants. Also coordinating the EU project LEGATO (Legumes for the <u>Agriculture of Tomorrow</u> (2014-2017)), aimed at increasing grain legume cultivation in Europe.

Use of translational genomics to identify genes important for legume seed development

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We have been using genomics approaches with *Medicago truncatula* as a tool to identify key genes determining seed yield and composition in Medicago and in closely related legumes. Analyses of the proteome and transcriptome of the component tissues of the developing seed revealed extensive compartmentalization of gene expression and metabolic activities (Gallardo et al, 2007). Using a TF (Transcription Factor) qRT-PCR platform (Verdier et al., 2008) the Affymetrix Gene Chip (Benedito et al, 2008), and more recently, Nimblegen arrays (Buitink et al. submitted), putative regulatory genes specific for each seed tissue were identified, along with putative target genes of transcription factors (TFs). These genes have been located on the *M. truncatula* genetic map and correlations between map positions of TF loci and QTLs for protein quantities and other seed phenotypes were detected. These correlations were confirmed in certain cases by the existence of similar QTLs at syntenic positions in pea. This approach has enabled us to attribute roles to two genes, both specifically expressed in the developing endosperm of *M. truncatula* and present in pea. One encodes a DOF class transcription factor, whose mutant phenotype severely affects endosperm development. The second gene encodes an endosperm-specific subtilase (*SBT1.1*), which affects final seed weight in both species (D'Erfurth et al, 2012). The importance of the endosperm in determining legume seed size and composition will be discussed.

The research leading to these results has received funding from the European Community FP7 grant agreements FP7 KBBE-613551, LEGATO, and FP7 KBBE-289562, ABSTRESS, from the ANR (QualityLegSeed and GenoPea), and from the Burgundy Regional Council.



Dr. Grusak is a Plant Physiologist at the USDA-ARS Children's Nutrition Research Center (CNRC), Houston, TX and a Professor of Pediatrics, Baylor College of Medicine. He joined the CNRC in 1990 to develop an interdisciplinary program to link crop science and production agriculture with human nutrition concerns. His research involves understanding the molecular mechanisms and regulation of nutrient transport within plants, with the long-term goal of enhancing the nutritional quality of plant foods for human consumption. Dr. Grusak serves on the Editorial Boards of the journals: Plant and Soil, Crop Science, Plant Foods for Human Nutrition, and Rice Journal. He serves on the Board of Directors of the Texas Academy of Science and is the 2015 President-Elect for the Crop Science Society of America. He was recently appointed as the Scientific Officer for the Office of Scientific Quality Review, which conducts peer review of all USDA-ARS research projects.

Nutritional Enhancement of Food Crops: Recent Conceptual Developments and Strategic Targets for Common Bean

Grusak, M.A.^{1*}. ¹USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX. *(mike.grusak@ars.usda.gov)

Food crops are an important source of nutritional and health-beneficial compounds for humans. Unfortunately, mineral deficiencies abound in certain population groups due to food insecurity or to the inadequate nutritional composition of staple foods. In recent years, crop scientists have realized that agriculture could be used to impact human health and have worked to enhance nutritional density in staple crops through breeding approaches or management strategies. Our group has focused on several grain legumes to understand the whole-plant dynamics and temporal patterns that control mineral allocation to seeds. We are interested in how the delivery of minerals to aerial tissues is controlled by root and soil processes, and in the crosstalk that occurs between shoots and roots. We also wish to know how mineral trafficking to seeds is regulated by processes in source tissues, which themselves require minerals. In this talk, results will be presented on whole-plant regional and temporal patterns of mineral partitioning in common bean, which are being used to assess where the rate-limiting steps are occuring in the trafficking of various minerals throughout the plant. We will discuss which processes might be the best targets for increasing seed mineral levels, and will provide data on the extent to which these enhancements might be achievable in light of current source tissue pool sizes. Lastly, we will present a framework for setting target levels and assessing dietary impact of a given enhancement strategy. This work was funded by USDA-ARS (Agreement 58-6250-0-008) and by USAID (Feed the Future Grain Legumes Project).

Keynote Speaker: Legume Mega Projects (ICLGG)



Kirstin Bett is Professor of Plant Breeding and Genetics in the Department of Plant Sciences, University of Saskatchewan in Canada. She teaches courses at the graduate and undergraduate level in plant breeding and plant genetics. She is currently responsible for a common bean breeding program and genomic and genetic research in pulse crops. Three of her pinto bean cultivars and a yellow bean cultivar are now being grown commercially in western Canada. She has also established a complementary genetics program that uses classical and molecular techniques to better understand the traits that lead to the development of superior pulse crop cultivars. This has included work in seed quality, disease resistance and cold tolerance and has extended to the use of wild species as a source of useful variability. She has been involved in the development of genomic resources for pulse crops and is currently leading the effort to sequence the lentil genome.

Lentil genome sequencing: establishing a comprehensive platform for molecular breeding

Kirstin Bett, Larissa Ramsay, Andrew Sharpe, Douglas Cook, R. Varma Penmetsa, Neha Verma, Melissa Wong, Crystal Chan, Albert Vandenberg, Allen VanDeynze, Clarice Coyne, Rebecca McGee, Dorrie Main, Jaroslav Doezel, Dave Edwards, Sukhijwan Kaur, Shiv Kumar Agrawal, Sripada Udupa

While global production of lentil (*Lens culinaris*) is on the rise, breeding programs are limited in their ability to implement marker-assisted selection (MAS) due to a lack of genomic resources. Large steps have been taken lately to develop SNP-based markers but the availability of a reference genome sequence will provide a basis for further crop improvement. With the common bean and chickpea draft genome sequences now available, it is time to tackle the larger pulse crop genomes such as lentil. Through funding from the Saskatchewan Pulse Growers we have been able to carry out large amounts of next-generation sequencing on CDC Redberry, a small red cultivar from Saskatchewan. An initial draft of 23x coverage produced scaffolds covering over half the genome (2.7 Gb of the expected 4.3 Gb) and recent additional 125x coverage is currently being assembled. Long reads based on PacBio sequencing are being used to assemble smaller scaffolds into larger assemblies. Genotype by sequencing (GBS) in key mapping populations is being used to anchor scaffolds as well as select individuals for resequencing. Collaborations with labs in Australia, Czech Republic, USA and ICARDA will facilitate further assembly and annotation of the genome as well as add to the growing database of genotypic diversity in the global lentil germplasm. The sequence data from the initial 23x coverage was sufficient for the identification of gene sequences for traits of interest in the lentil breeding program and derived SNP markers are now being used in routine MAS.

Keynote Speaker: Nitrogen Fixation and Plant Nutrition (IFLRC)



Ken Giller is Professor Plant Production Systems (<u>http://www.pps.wur.nl</u>), within *WaCASA* (the Wageningen Centre for Agroecology and Systems Analysis - <u>http://www.wacasa.wur.nl</u>) at Wageningen University. He leads a group of scientists with profound experience in applying systems analysis to explore future scenarios for land use with a focus on food production. Ken's research has focused on smallholder farming systems in sub-Saharan Africa, and in particular problems of soil fertility and the role of nitrogen fixation in tropical legumes, with emphasis on the temporal and spatial dynamics of resources within crop/livestock farming systems and their interactions. He is author of the standard text "*Nitrogen Fixation in Tropical Cropping Systems*" published in second edition in 2001. He leads a number of initiatives such as N2Africa (Putting Nitrogen Fixation to Work for Smallholder Farmers in Africa - <u>http://www.n2africa.org/</u>). Ken joined Wageningen University in 2001 after holding professorships at Wye College, University of London, and the University of Zimbabwe.

N2Africa: Putting nitrogen fixation to work for smallholder farmers in Africa

Ken E. Giller

Plant Production Systems, Wageningen University, P.O. Box 430, 6700AK Wageningen, The Netherlands.

N2Africa (<u>http://www.n2africa.org/</u>) is a large development-to-research project that focuses on tapping expertise from all around the world to ensure the best technologies find their way into the hands of smallholder farmers in Africa. The major output of the project is enhanced productivity, nitrogen fixation and production of the major grain legumes, common bean, groundnut, cowpea, soyabean, chickpea and faba bean is enhanced. We work across 11 countries in East and Central, West and southern Africa through a wide range of partnerships with research and development organisations.

Successful N₂-fixation by legumes in the field depends on the interaction: $(G_L \times G_R) \times E \times M$ that is (legume genotype × rhizobium genotype) × environment × management. Environment encompasses climate and soil stresses. Management includes aspects of agronomic management (use of rhizobial inoculum, mineral fertilizers, sowing dates, plant density, weeding). Although much legume research is focused on identifying best combinations of G_L and G_R , the E and M factors often override the potential of the legume/rhizobium symbiosis for N₂-fixation in the field. Our approach is to target nitrogen fixation technologies to 'socioecological niches' within the huge diversity of countries and agroecologies and farming systems in Africa.

In the second phase of N2Africa, which has just begun, we aim to increase inputs from N₂-fixation on more than 550,000 smallholder farms within the coming five years through: a) Increasing the area of land cropped with legumes; b) Increasing legume productivity through better agronomy and basal (P, K etc) fertilizer; c) Selecting and disseminating legume varieties with increased N₂-fixation; d) Selecting better rhizobium strains and promoting high quality inoculants; e) Linking farmers to markets and creating new enterprises to increase demand for legumes. N2Africa has already reached more than 250,000 farmers and the latest learning will be discussed.

Keynote Speaker: Biotic Stress and Plant Microbe Interactions (ICLGG)



Jens Stougaard is Professor of Molecular Biology and Genetics at Aarhus University and Director of the Centre for Carbohydrate Recognition and Signalling (CARB). Jens Stougaard leads a group studying genes regulating development of nitrogen fixing root nodules and mycorrhiza formation in legumes. Currently the mechanisms of Nod-factor perception, the function of receptors involved and the downstream signal transduction cascades are in focus. The plant model system used for this research is *Lotus japonicus* that is also used for investigating the long range signalling integrating root nodule development into the general developmental program of the plant. Genetics, genomics and biochemical methods are used to identify and characterise components of regulatory circuits. In order to improve the genetic analysis and to establish a system for reverse genetics, a large-scale insertion population based on the germ-line specific activity of the LORE1 retroelement is being established and made available to the community.

Bacterial infection of epidermal cells is controlled by a two-step recognition process

Stougaard, J.^{1*}. ¹Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10, DK-8000 Denmark. *(stougaard@mb.au.dk)

Development of root nodules in legumes in response to signals secreted from rhizobiais an example of a regulated bacterial infection process that is synchronised with an inducible organ formation. Lipochitooligosaccharides (Nod-factors), consisting of substituted b,1-4 N-acetylglucosamine (chitin) backbones are the major rhizobial signals triggering root hair deformation, initiation of the bacterial infection process and start of the cell divisions leading to formation of nodule primordia. An important determinant of bacterial recognition and host specificity is the interaction between Nod-factors and the plant receptors involved in signal perception and signal transduction initiating the plant developmental response. In *Lotus japonicus* there are seventeen LysM receptor kinases that could be involved in perception of Nod-factors or other chitin derived signal molecules. The possible role of these LysM type serine/threonine receptor kinases in plant-microbe interaction will be discussed. Biochemical experiments investigating the function of LysM receptors will be presented and the involvement of NFR1 and NFR5 receptor kinases in the earliest physiological and cellular responses will be illustrated. A second recognition step has recently been discovered and the molecular basis of this novel non-self recognition will be presented together with a model for two-step recognition of rhizobial bacteria at the initiation of infection.

Bek et al. (2010) Improved Characterization of Nod Factors and Genetically Based Variation in LysM Receptor Domains Identify Amino Acids Expendable for Nod Factor Recognition in *Lotus* spp. MPMI, 23, 58-66.

Broghammer et al (2012) Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. PNAS 109, 13859-14864.

Keynote Speaker: Biotic Stress and Plant Microbe Interactions (IFLRC)



Alain Baranger is a geneticist at UMR IGEPP Institute, INRA Rennes, France. He leads a research group focusing on efficiency and durability of the control of pathogen epidemics in legumes, including ascochyta blight and aphanomyces root rot in pea, using standard as well as molecular-based methods. This involves the exploration of variability in the host response (quantitative resistance), of diversity and evolution of pathogen populations (adaptation), and of epidemic processes. Current considered issues are the potential role of plant and canopy architecture traits to control epidemics through various processes (spore dispersion, microclimate, senescence), and the relative and complementary role of plant/canopy architecture and plant resistance genetic factors to reduce disease severity and effects.

Plant architecture and development to control aerial disease epidemics in legumes: the case of Ascochyta blight in pea

Baranger, A.^{1*}, Giorgetti, C.¹, Jumel, S.¹, Langrume, C.¹, Moussart, A.^{1,2}, Onfroy, C.^{1,2}, Richard, B.¹, Rivière, J.P.¹, Vetel, P.¹, and Tivoli, B.¹. ¹INRA, UMR1349 IGEPP, Le Rheu F-35653, France; ²UNIP-CETIOM, 11 rue de Monceau, CS 60003, F-75378 Paris, France. *(Alain.Baranger@rennes.inra.fr)

Plant and canopy architecture can contribute to the control of aerial diseases by creating a less conducive environment to their development. This control involves the modification of specific processes such as plant ageing and senescence, microclimatic conditions (leaf wetness duration, temperature and light penetration) and spore dispersal. Ascochyta blight in pea, mainly due to Didymella pinodes, is the most encountered and damaging aerial disease worldwide. Available resistance to this pathogen is scarce, partial, and is not always fully expressed in the field. Alternative strategies based on plant and canopy architecture and development modifications have shown that internode length and plant height, as well as leaf area index and senescence are key factors in Ascochyta blight epidemics. The genetic control of such and other architectural and developmental variables (earliness, stipule size, aerial biomass) relies both on some major genes and QTL that often colocalize with known factors controlling partial resistance components. We thus developed different biparental recombinant inbred line populations segregating for these major genes and QTL, as well as near isogenic lines from heterogeneous inbred families segregating within colocalizing QTL confidence intervals. These populations were screened in controlled conditions, where architecture and development are not likely to interfere with epidemics, to define whether these colocalizations were due either to linkage or to pleiotropic genetic effects. First insights showed that linkage effects are involved.

Keynote Speaker: Applied Genomics (ICLGG)



Dr. Rajeev Varshney, an Indian national and Principal Scientist (Applied Genomics) is serving ICRISAT as a Research Program Director, Grain Legumes and Director - Center of Excellence in Genomics. In addition to serving ICRISAT, Rajeev, in his dual appointment also served CGIAR Generation Challenge Program based in Mexico as Theme Leader for six years. Before joining ICRISAT, he worked at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany for five years.

Rajeev has a basic background in molecular genetics and possess more than 15 years research experience in international agriculture. The primary contribution of Rajeev Varshney includes genome sequencing of pigeonpea and chickpea and first generation of molecular breeding products in chickpea and groundnut in addition to large-scale genomic resources like molecular markers, transcriptome assemblies and QTLs for a range of traits in legumes.

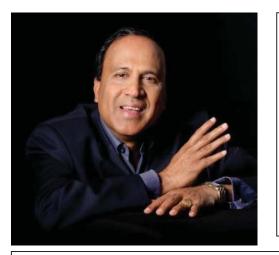
Rajeev has >200 publications in leading journals of international reputes including Nature (1), Nature Biotechnology (3), PNAS (1), etc., 10 edited books and Special Issues (as Guest Editor) for several journals to his credit. He has been a frequent invited speaker in several national/ international conferences including G-8 Conference on "Open Data for Agriculture", FAO conference on "Application of Biotechnologies in Developing Countries" and "Digital Design Meeting", chaired by Mr Bill Gates.

The 1000 Pulse Genome Sequencing Initiative: Connecting Genes to Traits

Varshney, RK.^{1*}. ¹International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. *(r.k.varshney@cgiar.org)

Chickpea (*Cicer arietinum*) and pigeonpea (*Cajanus cajan*) are playing a vital role in ensuring the nutritional food security in several countries of Asia and sub-Saharan Africa. Average productivity of these pulses is less than 1 t ha–1 due to their expousre to several biotic and abiotic stresses. Breeding efforts to elevate the yield levels and enhance crop productivity could not be achieved due to availability of limited genomic resources andlow level of genetic diversity present in the cultivated pools. ICRISAT genebank has >20,000 and >13,000 accessions of chickpea and pigeonpea, respectively. With an objective to 'explore this huge genetic diversity available to address the issue of low productivity and bottlenecks' associated with narrow genetic diversity, >1000 pulse genomes including 554 chickpea (reference set, elite varieties and parents of several mapping populations) and 526 pigeonpea (reference set, hybrid parental lines, wild species accession and parents of mapping populations) genomes were sequenced at 5X to 13X coverage. These genomes were aligned to their respective reference genomes for variant calling and structural variation analysis. Detailed analysis provided comprehenisve data on diversity features, gene loss, domestication and selection sweep. Multi-location phenotyping data for high priority traits for breeding is being assembled for undertaking genome-wide association studies. In brief, this initiative is expected to identify superior/novel alleles associated with the traits of interest for enhancing crop productivity of these pulse crops.

Keynote Speaker: Abiotic Stress and Crop Management (IFLRC)



Professor Kadambot Siddique is the Hackett Professor of Agriculture Chair and Director of The University of Western Australia's Institute of Agriculture. He has 30 years' experience in agricultural research, teaching and management in both Australia and overseas. He has developed a national and international reputation in agricultural science especially in the fields of crop physiology, production agronomy, farming systems, genetic resources, breeding research in cereal, grain and pasture legumes and oilseed crops. Professor Siddique's publications are considered as key papers in the above fields and are widely cited. As a result of Professor Siddique's personal research and with others with whom he collaborates, Australia has become one of the major grain legume exporting nations in the world. His pioneering research on chickpea has contributed enormously to the Australian chickpea industry which is currently valued at more than \$300 million per annum.

Professor Siddique is winner of the **Western Australian Year of the Award 2014** (Professions Category) for his contribution to agricultural science and farming community. In September 2013 Professor Siddique was honoured with a prestigious **Dunhunag Award** by China's Gansu Provincial Government for his outstanding contribution to research and leadership within Gansu Province, especially at Lanzhou University. In 2013 Professor Siddique was elected as a **Fellow of the Australian Agricultural Institute** (FAAI) and the citation recognised his outstanding contribution to professional agriculture. In 2011 Professor Siddique was made **Member of the Order of Australia** (AM) in Queen's Birthday Honours List. The citation recognised his lifetime's work in advancing agricultural science as an academic and researcher in the area of crop improvement and agronomy and through contributions to professional associations. In 2005 he was elected as a **Fellow of the Australian Academy of Technological Sciences and Engineering** (FTSE). In 2009 he received a gold medal and citation from the former **President of India, Dr A.P.J. Abdul Kalam**, for his international contribution to Australian agricultural science and the industry. Professor Siddique received **the prestigious "Urrbrae Memorial Award"** for his contribution to Australian agricultural science and the industry. Professor Siddique has published more than 250 scientific papers and book chapters. Professor Siddique is on the Editorial Board of a number of international scientific journals. He has also trained numerous Honours, MSc and PhD students. He is a visiting Professor in a number of overseas universities.

ABIOTIC STRESS IN COOL SEASON GRAIN LEGUMES: GENETIC AND AGRONOMIC APPROACHES

Siddique, K.H.M.^{1*}, Pang, J.^{1,2}, and Khan, T.N.¹. ¹The UWA Institute of Agriculture, University of Western Australia; ²School of Plant Biology, University of Western Australia. *(kadambot.Siddique@uwa.edu.au)

Among the cool season grain legume species, chickpea, field pea, lentil and faba beans are the most important. They are grown on a range of soil types and environments experiencing various abiotic stresses. Drought at early developmental stages, flowering and pollination and pod andseed set stages may adversely affect yield. High temperatures often combined with drought may also inhibit pollination and pod/seed development. Excess water while causing transient water logging damage may also lead to salinity in the soil. Low temperatures may lead to freezing injury at all stages and less than optimum temperature at flowering affects pollination and pod set. Oftenenvironmental factors interact, e. g. high temperature and drought; salinity and waterlogging. Grain legumes vary in their sensitivity and this may help to choose the appropriate species to address the abiotic constraint identified. Progress has been made in understanding physiological and genetic mechanism and developing tools for selection of key traits for drought resistance and salinity tolerance but such research on low temperature/freezing tolerance or water logging tolerance is sparse. Agronomic management such as time of sowing, no-till technology, weed management and adequate fertilizer application has also been successfully applied to alleviate abiotic stress and their agronomic management continues to offer formidable challenges.

Concurrent Session Abstracts

Monday, July 7, 2014

Fundamental and Applied Genetics and Genomics

ICLGG Fundamental Genomics

Chair: Doug Cook (University of California Davis, USA)

11:00-11:30 Presenter: Scott Jackson (Univ. of Georgia, USA)

Genetic and epigenetic variation in legumes: focus on Glycine max and Phaseolus vulgaris.

Jackson, S.A.^{1*}, Kim, K-D.¹, ElBaidouri, M.¹, Schmitz, R.¹, Libault, M.², Schmutz, J.³, Qiu, L.⁴, and Li, Y-H.⁴. ¹University of Georgia; ²University of Oklahoma; ³Hudson Alpha Institute for Biotechnology; ⁴Chinese Academy of Agricultural Sciences. *(sjackson@uga.edu)

Reference genome sequences are available for soybean (Glycine max) and common bean (Phaseolus vulgaris). However, these reference genome sequences do not represent the array of variation contained within a species. In order to explore this variation, we created a pan-genome for undomesticated soybean (Glycine soja) and resequenced more than 100 landraces and wild relatives of common bean. Both data sets provide differing levels of insight into sequence and structural variation within a species and provide a framework for examining genetic variation as a tool for crop improvement. In addition, epigenetic variation, that cause by DNA packaging, is a potential source of variation that is being explored to understand its evolution and role variation that is available, or has been used for improvement.

11:30-11:50 Presenter: Stig Andersen (Aarhus University, Denmark)

Lotus japonicus natural variation and LORE1 insertion mutants

Gupta, V.¹, Malolepszy, A.¹, Shah, N.¹, Hirakawa, H.², Fukai, E.³, Jin, H.¹, Mun, T.¹, Urbanski, D.¹, Kawaharada, Y.¹, Markmann, K.¹, Sandal, N.¹, Umehara, Y.³, Soyana, T.³, Miyahara, A.³, Schierup, M.⁴, Hayashi, M.³, Busch, W.⁵, Sato, S.^{2.6}, Stougaard, J.¹, and Andersen, S. U.^{1*}. ¹Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10, DK-8000 Aarhus C, Denmark; ²Kazusa DNA Research Institute, 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818 Japan; ³Division of Plant Sciences, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan; ⁴Bioinformatics Research Centre, C. F. Møllers Allé 8, Aarhus University, DK-8000 Aarhus C, Denmark; ⁵Gregor Mendel Institute of Molecular Plant Biology, Dr. Bohr-Gasse 3, 1030 Vienna, Austria; ⁶Graduate School of Life Sciences, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai, 980-8577, Japan. *(sua@mb.au.dk)

We are in the process of transforming the genomic resources of the model legume *L. japonicus* to allow QTL and genome-wide association studies of multigene inherited traits to be combined with efficient validation by reverse genetics. These approaches will facilitate study of a wide range of traits, including interactions with symbionts and other microbes, but require a complete and well-annotated genome, a catalog of natural variation, accurate phenotyping methods, and a comprehensive mutant collection. To provide the genotype data needed, we have updated the genome assembly and annotation and re-

sequenced 124 Japanese *L. japonicus* accessions. In order to generate accurate phenotype data, we have developed high-throughput image-based methods for root growth trait quantification. The results of genome-wide association studies based on combined use of these datasets will be described. Testing of the resulting candidate genes is greatly facilitated by access to LORE1 insertion mutants, and the status of the LORE1 collection, which now includes more than 340,000 annotated insertions, will be presented (www.carb.au.dk/lore1).

11:50-12:10 Presenter: Greg Perry (University of Guelph, Canada)

Using SNP genotyping to identify regions of *Phaseolus acutifolius* introgression in OAC-Rex, a CBB resistant variety of *Phaseolus vulgaris*

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OAC-Rex was the first variety of dry bean released in North America that was resistant to Common Bacterial Blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli*. Although some resistance to CBB has been detected in the vulgaris germplasam, strong resistance has only been achieved using interspecific crosses between P. vulgaris and species such as *Phaseolus coccineus*, and *Phaseolus acutifolius*. In the case of OAC-Rex, the CBB resistance was the result of an interspecific hybridization with the *P. acutifolius* line Pl440795. As part of the Applied Bean Genomics and Bioproducts Project, the genomic sequence for OAC-Rex and Pl440795 is being sequenced using the Illumina HiSeq platform. Although the full-length chromosomal sequence is not available at this time, contigs and scaffolds allow us to interrogate both lines using BLAST. Using the SNP markers from the BeanCAP project, we identified over 6000 markers present in OAC-Rex, and 3800 in Pl440795. Markers which were shared between the two lines were examined for alleles which could be considered as acutifolius specific by comparing them to G19833, an Andean *P. vulgaris* line, whose genomic sequence has been previously published. By identifying regions with potential *P. acutifolius*-derived sequence, we will be able to better understand the rearrangement of the *P. vulgaris* and *P. acutifolius* genetic information during the interspecific cross.

12:10-12:30 Presenter: Stephan Schröder (North Dakota State University, USA)

Optimization of Genotyping by Sequencing (GBS) Data in Common Bean (Phaseolus vulgaris L.)

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The standard version of the Genotyping by Sequencing (GBS) method uses a relatively large fragment pool and is typically sequenced at low coverage (1X). This often results in misscoring of heterozygotes as homozygotes and a high rate (\geq 30%) of missing data points. Common bean has a haploid genome size of 550 Mb. We are interested in improving quality and coverage of GBS data in order to: i) accurately estimate genetic diversity in a drought diversity panel and ii) find associations with traits of agronomic importance using a Genome-Wide Association approach. With these two considerations in mind, we developed an improved GBS method, which utilizes an *in silico* digest of the bean genome to predict

fragment density and length, a double digest of genomic DNA, ligation of a barcoded and a common Yadapter, and DNA fragment size selection to achieve a reduced fragment pool that can be sequenced at higher coverage. In our pilot study consisting of 6 diverse Middle American dry bean lines, we obtained reads that were present at a coverage \geq 3X in all six lines. More than 14,000 high quality SNPs were identified, with an average of one SNP every 36 Kb and the maximum distance between adjacent SNPs of 1.75MB. These preliminary results provide us with a high marker density which will enable us to address the questions mentioned above.

IFLRC Applied Genetics & Genomics

Chair: Tom Warkentin (University of Saskatchewan, Canada)

11:00-11:30 Presenter: Noel Ellis (CGIAR, Hyderabad, India)

Shifting the focus: populations, genetics and genomics

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For many legume species whole genome sequence information is either available or; being generated, this resource can provide many tools for genome analysis. Extensive germplasm collections for legumes, held in a variety of institutions, have been characterised in terms of their genomic diversity enabling targeted access to accessions tailored to a variety of needs. These can be accessed, for example, by searching for maximally different genomes, or by comprehensive searches within a sub-population. The generation of transgenic plants is also feasible in many legume species, as is the analysis of gene function in transient assays such as VIGS. All of these should provide a rich resource for understanding and deploying appropriate genes in breeding programmes. However, two additional resources are required: systematic mutant populations and consensus genetic linkage maps.

Systematically curated mutant populations provide an alternative to VIGS and transgenesis for analysis of gene function. These have been generated in several species, but are not yet available for all legume crops.

Genetic maps are not made redundant by genome sequence: they describe the distribution of alleles in populations and this is essential for connecting genotype and phenotype. In order to exploit mutant populations and genomic tools we need robust genetic analysis. The efficient application of genomics to breeding requires the sharing of both genotypic and phenotypic data: a lesson to be learnt from the rapid progress in genomics that was driven by data sharing. The time is ripe for a re-emphasis on the use of genetics to dissect and understand traits in terms of their discrete determinants.

11:30-11:45 Presenter: Rachit K. Saxena (ICRISAT, India)

Whole genome re-sequencing based classification of heterotic pools for accelerating hybrid breeding in pigeonpea

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Hybrid technology has potential to elevate the yield levels in pigeonpea. Recently, the world's first grain legume hybrid of pigeonpea ICPH 2671 has been released for commercial cultivation in India, which showed 47% yield advantage over the check varieties. To develop such hybrids, breeders make thousands of random crosses between cytoplasmic male sterile (CMS) lines and tester lines. Genomic diversity along with the phenotyping data have been used for predicting best possible combinations and defining heterotic pools in many crop species such as maize, rice, sunflower and rapeseeds. In order to define heterotic pools in pigeonpea, a set of 104 parental lines (10 CMS, 12 maintainers and 82 restorers) have been re-sequenced following whole genome re-sequencing (WGRS) approach. WGRS yielded 511 GB sequence data with the coverage ranging from 5X to 10X. A total of 3.4 million SNPs could be identified across 104 lines, while comparing individual genotypes with the reference genome and SNPs ranged from a minimum of 15,388 to a maximum of 84, 851. Structural variations such as copy number and presence/ absence variations are being identified. In parallel, these parental lines are being used to develop testcrosses in factorial mating design. F1 hybrids along with parental lines will be phenotyped for yield and yield related traits. In brief, the availability of genome-wide SNP variations combined with the phenotypic data should provide clues on candidate genomic regions associated with yield and yield related traits as well as those associated with heterosis and hetereotic pools in pigeonpea for accelerating hybrid improvement.

11:45-12:00 Presenter: Ping Wan (Beijing University of Agriculture)

A high-density SNP genetic map and QTL mapping for yield and other agronomic traits in adzuki bean (*Vigna angularis*)

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Cultivar ass001, wild adzuki bean CWA108 and one hundred fifty F2 individuals derived from the cross of them were sequenced by RAD-seq technology. In total, 133.93G base sequences and 379024 SNPs were obtained. 3617 segregated SNPs were used to mapping, a SNPs genetic map of adzuki bean was firstly constructed. The genetic map is composed of 1571 SNP markers, spanned over the distance of 1031.171 cM with an average distance between markers of 0.656 cM and covered 11 linkage groups.

The important agronomic trait QTLs of bloomin and ripening time, leaf length and width, stem tickness, pod width, 100-seed weight and the number of seeds per pod were mapped by SNPs markers. Three flowering time (FLD) QTLs on chromosome 3, 4 and 5 explained 8.5% 10.6% and 9.4% of phenotypic variance. Five QTLs controlling mature stage were mapped on chromosome 2, 3 and 4, and phenotypic variances were 12.9%, 9.9%, 15.9% ,18% and 11.2%. QTLs of FLD qFLD50 4, maturity qPDDM 50 4 and qPDDM100 4 have the same co-segregated SNP marker s58_238422. QTLs of leaf length, stem tickness and pod width were mapped on different region of chromosome 10, and explained 12.7% 13.7% and 11.3% of phenotypic variance. Two leaf width QTLs were mapped on chromosome 2 and 6, which explained of 7.3% and 7.2%. phenotypic variance. Two 100-seed weight (SD100WT) QTLs of chromosome 6 and 10 were identified with the phenotypic variance of 13.3% and 11.0%. QTLs of

SD100WT 10 and stem tickness STT 10 have the same co-segregated SNP marker s41_311101. one major QTL of seed number per pod SDNPPD 6 was mapped on chromosome 6, explained 11.9% phenotypic variance. The QTLs of SDNPPD 6, 100-seed weight SD100WT 6, leaf width LFMW 6 located in common SNP marker interval of s768_70977 and s85_114079, and were co-segregated with s130_56874.

This work was supported by fund from National Natural Science Foundation of China (Project Number 31071474)

12:00-12:15 Presenter: Trupti Joshi (University of Missouri, Columbia, USA)

Next Generation Resequencing of Soybean Germplasm for Trait Discovery

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With the advances in next generation sequencing (NGS) technology and significant reduction in sequencing costs it is now possible to sequence large sets of crop germplasm and generate whole genome scale structural variations and genotypic data. In depth informatics analysis of the genotypic data can provide better understanding of the links with the observed phenotypic changes. This approach can be used to further understand and study different traits for the improvement of crops by design.

We have conducted resequencing of several soybean germplasm lines selected for major traits including oil, protein, soybean cyst nematode resistance (SCN), abiotic stress resistance (drought, heat and salt) and root system architecture. We have done bioinformatics analysis and identified SNPs and insertion, deletions using GATK3.0 software. We have also conducted copy number variations (CNV) analysis and SNP annotations with SnpEff. We conducted 25 genomes case study for analysis of SCN resistance and classified them into four different categories of resistance and susceptibility levels. GWAS analysis identified major SNPs associated with the phenotypic changes between these lines. We have performed linkage disequilibrium and haplotype analysis using Haploview. We have also applied generalized linear models (GLM) and mixed linear models (MLM) using TASSEL for identifying SNPs significant for phenotypic changes.

Analysis was conducted using XSEDE as the computing infrastructure, iPlant as the data and cloud infrastructure, and the Pegasus workflow systems to control and coordinate the data management and computational tasks. All data including GWAS, SNP annotations can be accessed through Soybean Knowledge Base (SoyKB) at http://soykb.org.

12:15-12:30 Presenter: Gilles Boutet (INRA, Rennes, France)

SNP discovery in pea: A powerful tool for academic research and breeding

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Pea has a 4.3 Gb complex genome for which only limited genomic and sequencing resources are available to date. In order to answer to an increasing demand from French breeders for a breakthrough in Marker Assisted Selection, we carried out a massive development of markers in pea.

We sequenced eight cDNA normalized libraries from genotypes representative of modern pea breeding material, assembled a large set of 10,000 cDNA contigs, and identified over 35,000 reliable SNP markers. A SNP subset was genotyped with the Golden Gate assay to generate a high density reference composite genetic map covering 1255 cM and comprising 2070 markers including 1340 newly developed SNPs, anchored to the *M. truncatula* physical map. Developed SNPs furthermore showed efficiency in structuring diversity in a collection of pea cultivars, even using a proposed reduced subset of 297 most informative SNPs (Duarte *et al.*, 2014).

A complementary approach of SNP "genotyping by sequencing" ran on genomic DNA libraries from a 48 RILs mapping population. It has developed to date more than 100,000 SNPs, among which approximately 60,000 will undergo mapping (unpublished).

This work gives a comprehensive knowledge for the selection of choice subsets of SNP markers useful for polymorphism, mapping and hierarchical information purposes. These new resources publicly delivered by the PEAPOL project will thus help as tools in cumulating alleles at QTLs for traits of interest, directing the creation of new pea ideotypes adapted to various climates and cropping systems, with stabilized and high yields.

Duarte et al. (2014) BMC Genomics 15:126

ICLGG Evolution

Chair: Sachiko Isobe (Kazusa DNA Research Institute, Japan)

14:00-14:30 Presenter: Peter Tiffin (University of Minnesota, USA)

Targets of selection in the Medicago genome

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Population genomic data provide opportunities to identify the genes that contribute to naturally occurring phenotypic variation and to characterize how selection has shaped the evolution of those genes. I will present results from three series of analyses that made use of full genome sequence data from more than 200 accessions of M. truncatula. These analyses: i) identify targets of selection and how expression, genomic location, and recombination affects genome-wide diversity, ii) characterize the role of local adaptation, and iii) compare whether selection has acted differently on genes identified through forward genetic screeens and association genetic analyses. The results from these analyses identify legume genes that may be most likely to contribute to phenotypic variation (and thus most amenable for crop improvement), the evolutionary history of nodulation genes, and the evolutionary stability of symbiotic interactions.

14:30-15:00 Presenter: Maren Friesen (Michigan State University, USA)

Symbiotic Dimensions of Trifolium Biodiversity

Maren L. Friesen¹, Sharon Y. Strauss², Brain L. Anacker², Joseph P. Dunham³, Prateek Shetty¹. 1: Department of Plant Biology, Michigan State University; 2: Department of Evolution and Ecology, University of California, Davis; 3: Section of Molecular and Computational Biology, University of Southern California

Understanding the biodiversity of natural plant communities has twofold relevance for agriculture. First, this diversity underlies the genetic variation drawn upon by breeders. Second, plants in both ecological and agronomic communities compete with one another for resources and structure the soil microbial communities important for plant performance. Diverse North American Trifolium-Rhizobium communities are an exemplar system in which the roles of positive and negative feedbacks in coexistence can be dissected at molecular, functional, and taxonomic levels. We find that plants make more large nodules in their home soils and that nodulation correlates with performance, demonstrating that rhizobia are an important part of the niche. Competition experiments show that plants allocate more to nodules when competing against members of their own species, suggesting that symbiotic interactions play a role in species coexistence. Nodule genotyping shows widespread sharing of rhizobium strains across Trifolium species with the majority of structuring occuring between different soils rather than species. Using 16S amplicon sequencing, we find that nodules harbor a small but diverse community of bacteria that is largely conserved; however, species structure this community to a small extent as does the soil from which they presumably originate. A de novo transcriptome assembly of one species is underway; preliminary results suggest that a high fraction of transcripts belong to root-colonizing fungi and thus fungal members of the microbiome may also play key roles in performance. The overarching goal of this work is to bridge organismal and community scales to predict coexistence and inform variation in biological nitrogen fixation.

15:00-15:20 Presenter: Steven Cannon (USDA-ARS)

Multiple incidents of polyploidy and nodule evolution near the origin of the legumes

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As part of the Thousand Plant Transcriptomes (1kp) project, we have evaluated the transcriptomes and genomic proteomes from 20 diverse legumes and 16 outgroup species. We reconstructed and analyzed thousands of phylogenetic trees calculated from transcriptomes and genomic proteomes to identify patterns consistent with whole genome duplications (polyploidy events). We find that the papilionoid whole genome duplication (WGD) occurred in the common ancestor of the entire Papilionoideae. The earliest diverging lineages of the subfamily include both non-nodulating and nodulating taxa. Among the latter are the familiar lineages of the subfamily: genistoids (e.g. lupin) and dalbergioids (e.g. peanut), phaseolids (e.g. beans), galegoids (e.g. clovers). Thus, the ancestral WGD likely influenced the evolution of all papilionoid species. We also find evidence for several independent WGDs near the base of other major legume lineages: in the Mimosoid-Cassiinae-Caesalpinieae (MCC) clade (the classical Mimosoideae clade and elements of the caesalpinioid grade that includes it), and in the two other sampled clades (Detarieae and Cercideae) that comprise the remainder of the caesalpinioid grade. We note that nodulation arose in

two clades that experienced WGD near their origins (the MCC and Papilionoid clades), but also that there are numerous non-nodulating lineages in both clades.

15:20-15:40 Presenter: Elisa Bellucci (Università Politecnica delle Marche, Ancona, Italy)

Decreased nucleotide and expression diversity and modified co-expression patterns characterize domestication in the common bean

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Using RNA sequencing technology and *de novo* transcriptome assembly, we compared representative sets of wild and domesticated accessions of *Phaseolus vulgaris* from Mesoamerica. RNA was extracted at the first true-leaf stage, and *de novo* assembly was used to develop a reference transcriptome; the final dataset consists of ~190,000 SNPs from 27,243 contigs in expressed genomic regions. A drastic reduction in nucleotide diversity (~60%) is evident for the domesticated form, compared to the wild form, and almost 50% of the contigs that are polymorphic were brought to fixation by domestication. In parallel, the effects of domesticated accessions demonstrate similar seminal network properties, they show distinct community structures that are enriched for different molecular functions. After simulating the demographic dynamics during domestication, we found that 9% of the genes were actively selected during domestication. We also show that selection induced a further reduction in the diversity of gene expression (26%) and was associated with five-fold enrichment of differentially expressed genes. While there is substantial evidence of positive selection associated to domestication, in a few cases, this selection has increased the nucleotide diversity in the domesticated pool at target loci associated to abiotic stress responses, flowering time, and morphology.

IFLRC Applied Genetics & Genomics; Disease Resistance Chair: Ashutosh Sarker (ICARDA, India)

14:00-14:15 Presenter: Janila Pasupuleti (ICRISAT, India)

Identification of introgression lines with superior pod yield and improved rust resistance in peanut (*Arachis hypogaea* L.)

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A major QTL explaining up to 82.62% of phenotypic variation for rust resistance was introgressed into three popular peanut varieties (TAG 24, ICGV 91114, JL 24) using marker-assisted backcrossing (MABC) approach. The donor parent, GPBD 4 derived its resistance from one of its interspecific parent, ICGV 86855 (CS 16), which in turn received from a diploid wild species, *Arachis cardenasii*. Four linked markers (IPAHM103, GM2079, GM1536, and GM2301) present in the QTL region were employed for foreground selection.

Preliminary yield evaluation of 51 introgression lines (ILs) (17 of TAG 24, 13 of ICGV 91114 and 21 of JL 24) along with parents and checks showed significant differences among the lines for yield parameters and rust resistance. The pod yield of ILs was up to 56-96% higher than recurrent parents. The superior pod yield in ILs is in part attributed to pod yield protection offered by resistance. The ILs were resistant to rust with a disease score of 2.0 or 2.5 at 90 DAS (on 1-9 scale) similar to donor parent, while the recurrent parents had higher score (5.0 in TAG 24, 6.5 in ICGV 91114, 7.0 in JL 24). Disease progress from 75 to 90 days was slower in ILs. The differences were not significant for days to flowering between ILs and their respective recurrent parents. Combining disease resistance and early maturity in peanut varieties is the most significant outcome. Further evaluations and promotion of promising ILs to multi location evaluation will facilitate nominations to varietal release trials.

14:15-14:30 Presenter: Ehsan Sari (University of Saskatchewan)

Genotype-dependent interaction of lentil lines with Ascochyta lentis

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Previous genetic studies suggested that resistance genes in the well-known ascochyta blight resistant lentil lines CDC Robin, ILL 7537, 964a-46, ILL 1704 are non-allelic. To understand how different resistance genes manifest resistance in these genotypes, cellular and molecular defense responses were compared after inoculation with the causal pathogen *Ascochyta lentis*. Histological tests suggested that cell-death inhibition might be mechanism of resistance in CDC Robin. By contrast, limited colonization by *A. lentis* of epidermal cells of 964a-46 was suggested as a mechanism of resistance in this line. Comparing the expression of key genes in the major signaling pathways of CDC Robin and 964a-46, the salicylic acid signaling pathway was only triggered in 964a-46. The jasmonic acid pathway was triggered in both resistant genotypes, however at a lower expression level in 964a-46 than in CDC Robin. These observations confirmed the existence of separate ascochyta blight resistance mechanisms in different lentil lines supporting the hypothesis of different resistance genes.

14:30-14:45 Presenter: Judith Lichtenzveig (Curtin University, Australia)

Pathogenomics of the Didymella spp. causal agents of Ascochyta blights in legumes

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The *Didymellaceae* family includes some of the most important pathogens of legume crops: the causal agents of Ascochyta blight in chickpea, pea, lentil, and faba beans among others (Aveskamp et al 2010). Despite their substantial economic impact, little is known about the molecular aspects of pathogenicity in these closely related species. At this meeting, the first genome assemblies and comparative analyses of eight legume pathogens, Ascochyta rabiei (chickpea), Peyronellaea pinodes (syn. Mycosphaerella pinodes), P. pinodella, A.pisi and Phoma koolunga (field pea), A. lentis (lentil), A. fabae (faba bean) and Phoma medicaginis (pathogen of the model legume Medicago truncatula) will be presented and discussed. Untrained *ab-initio* annotation resulted in the identification of 9,000-12,000 genes per species, of which 91-97% are complete gene models. The sequence data is serving to: pinpoint areas of synteny and clusters of genes associated with reproduction and adaptation; identify pathogenicity-related genecandidates through proteomic, transcriptomic and in silico comparative analyses; and design DNA makers for diagnostics and studies in population structure. Among the pathogenicity related genes, we have identified potential necrotrophic effectors that seem to operate similarly to those found other fungal pathogens of the order of the Pleosporales. Detailed understanding of the molecular mechanism involved in fungal adaptation in general, and pathogenicity in particular, is facilitating the development of novel tools and strategies in crop protection.

This project is co- funded by Grains Research and Development Corporation and Curtin University.

14:45-15:00 Presenter: Jagmeet Kaur (Punjab Agricultural University, Ludhiana, India)

Moisture stress induced changes in metabolites and cellular functions of chickpea (*Cicer arietinum* L.) genotypes

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The present investigation was aimed to study moisture stress induced changes in metabolites and cellular functions in *in vitro* identified tolerant (GL28151, RSG963, PDG3) and sensitive (GL22044, GNG1861, PBG1) chickpea genotypes. Moisture stress was induced during crop development by restricting irrigation at different growth stages viz. one pre-sowing irrigation (WSVFP), restricted irrigation at flower initiation (WSF), restricted irrigation at pod-initiation (WSP). Cellular functions (leaves) viz membrane permeability index, relative water content and lipid peroxidation and osmolytes (seeds) viz. total soluble sugars, starch, sucrose, proline, nitrogen content along with protein profiling were estimated in under control and stressed conditions. Antioxidative enzymes (peroxidase, catalase, superoxide dismutase and glutathione reductase) were assessed in seeds at pod filling stage. RSG963 was the most tolerant genotype in terms of minimal damage caused to cellular functions and attainment of maximum yield under stress conditions whereas GL22044 emerged as highly sensitive genotype with disruption in cellular functions and minimum yield. The least deterioration of cellular functions and higher accumulation of osmolytes, nitrogen and enhanced activities of anti oxidative enzymes in RSG963 might be responsible for rendering tolerance to moisture induced stress. The pronounced cellular damage and lesser alleviation in the content of osmolytes, nitrogen and activities of antioxidant enzymes was observed in sensitive genotype GL22044 under stress treatments. High molecular weight protein bands were found to be either absent or of low intensity in sensitive genotypes (GL22044, GNG1861 and PBG1) under severe stress treatment (WSVFP), sown with one pre-sowing irrigation.

15:00-15:15 Presenter: Renu Singh (CCS Haryana Agricultural University, India)

Mapping micronutrients using recombinant inbred lines (RILs) in Mung bean (Vigna radiata L.)

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Malnutrition, particularly among women, children and adolescents living in developing world, is an emergency that needs immediate attention. Vegetarian Indian diets are gualitatively deficient in micronutrients such as iron, calcium, vitamin A and zinc, which have direct impact on growth, metabolism and reproduction in human. One of the methods to mitigate micro-nutrient deficiencies is to develop food crop cultivars with higher levels of essential micro-nutrients. As such, the food legumes are of major nutritional importance, especially in developing countries, because they have high protein contents with biological values. Mung bean is one of the major food legumes consumed by people in Indian sub-continent. As an initial step, available mung bean genotypes were assessed for genetic diversity of selected micro-nutrient and protein contents. Marker analysis (AFLP & ISSR) shows moderate genetic diversity ranging from 65 to 87 %. Chemical analysis showed a fair range of micro-nutrient and protein variation (Fe content ranging from 1.6 to 9.3 mg/100g; Zn content ranging from 1.5 to 3.9 mg/100g and total protein content ranging from 21.1 to 30 %). Fe and Zn content showed a positive correlation (r = 0.469) along with fair heritability values (h2 = 0.259 for Fe and 0.727 for (Zn). The existing genetic diversity provides an opportunity to make indirect selection for both the traits. Results of RIL analysis are being used for developing new mung bean lines with higher contents of these micro-nutrients. The micronutrient content varied between individuals of the populations and values show transgressive segregation, but there was no significant association between the markers and the phenotypic traits.

15:15-15:30 Presenter: Larn McMurray (SARDI, Adelaide, Australia)

Improved weed control in Australian pulse production through advancements in genetic and agronomic research

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Australian winter pulse production of chickpea, lentil, faba bean and field pea is in excess of 1.0 M ha annually and offers rotational and cash crop benefits to growers (1). Successful and profitable pulse production is reliant on the use of modern, innovative and broad acre mechanized systems. Weeds are a major production constraint in these systems due to inherent low levels of crop competitive ability, limited availability of registered herbicides, low levels of crop herbicide safety and the increasing occurrence of herbicide resistant weeds (2). Pulse Breeding Australia and the Southern Region Pulse Agronomy

program are attempting to address these limitations in pulses through combining outputs from both breeding and agronomic research. This presentation will outline recent progress made by these programs in the areas of novel herbicide tolerance development (imidazolinone and metribuzin), agronomic herbicide management strategies for improved weed control and the development of varieties better suited to the agronomic practice of "crop-topping" herbicide resistant weeds. It will also discuss the current limitations to effective weed control in the Australian system, the current use of innovative agronomic production methods to improve weed control and future research and regulatory needs required in this area to help sustain Australian pulse production.

ICLGG Development

Chair: Sachiko Isobe (Kazusa DNA Research Institute, Japan)

16:00-16:30 Presenter: Catherine Rameau (INRA, Versailles , France)

Strigolactones and other long distances signals regulating shoot branching

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Shoot branching results from the tight regulation of axillary bud outgrowth at most leaf axils. For each axillary bud along the stem, endogenous and environmental signals (e.g. light, nutrition status) are integrated for maintaining the bud in a dormant state or inducing its outgrowth. The use of pea branching mutants and grafting experiments demonstrated the existence of a novel signal acting as a branching inhibitor, now identified as strigolactones (SL). Here several advantages of pea as a model species will be presented for elucidating several aspects of SL signaling pathway. Its simple architecture with easy access to axillary buds allows both precise phenotyping and comparison of shoot branching in the different mutants screened by forward or reverse genetics (TILLING). The possibility to apply SL onto the pea plant in several ways is very powerful for Structure-Activity-Relationships studies and for the use of labelled SL. Tritiated-labeled SLs and fluorescent SLs are being synthesized and their fate within the plant is analyzed. Different types of grafting are very easy to perform in pea for identifying and characterizing long distance signals. Evidences for further long distance signals, with a strong influence on shoot branching will be shown here, such as the *RMS3*-dependent graft-transmissible signal which can partially suppress branching, independently of SLs. The cloning of this gene demonstrates that *RMS3* encodes an α/β -hydrolase identified as the SL-receptor and very likely moving in the phoem sap.

16:30-17:00 Presenter: Jim Weller (University of Tasmania, Australia)

Genetic control of flowering in temperate legumes

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We are using a comparative approach to explore the genetic network controlling flowering time across a number of temperate legumes. We have performed comprehensive isolation/annotation of floweringrelated gene families in several legume species and are identifying genes with a role in flowering processes using forward and reverse screening and analysis of natural variation. In pea, we have cloned seven photoperiod-response loci. These include the SN, DNE, HR and PPD loci, which all inhibit flowering through a primary effect on circadian clock function. The PHYA photoreceptor, the GIGANTEA ortholog LATE1, and the newly identified LATE2 gene all promote flowering specifically under long days. Progress on understanding the interactions of these genes, and on the isolation of other flowering time loci will be reported. Systematic analysis of the six-member FT family of floral integrators in pea and Medicago has shown that specific *FT* genes are targets of photoperiod and vernalization regulation and have distinct expression patterns and developmental roles. In addition, QTL analyses of flowering time in several other legumes also implicate *FT* genes in control of flowering time. Overall, our results suggest that both clock and FT genes are important in legume flowering time adaptation, and that both groups of genes affect not only flowering time but also other aspects of development including growth habit. A better understanding of these genes should accelerate work on flowering and related traits across a wide range of legume species.

17:00-17:20 Presenter: Jianxin Ma (Purdue University, USA)

Molecular Basis of Soybean Stem Architecture

Ping, J.¹, Liu, Y.¹, Sun, L.¹, Zhao, M.¹, Nguyen, H.², Li, Y.³, Qiu, L.³, Nelson, R.L.⁴, Clemente, T.E.², Specht, J.E.², and Ma, J.^{1*}. ¹Department of Agronomy, Purdue University; ²Department of Agronomy and Horticulture, University of Nebraska; ³Institute of Crop Sciences, Chinese Academy of Agricultural Sciences; ⁴Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, US Department of Agriculture–Agricultural Research Service, and Department of Crop Sciences, University of Illinois. *(maj@purdue.edu)

Soybean (*Glycine max*) stem growth habit is a critical agronomic trait that affects the plant's yield potential and adaptability. Based on the timing of the termination of apical stem growth, soybeans can be classified into indeterminate, semi-determinate, and determinate types. Similar to *Arabidopsis*, the wild soybean (*Glycine soja*) and many cultivars exhibit indeterminate stem growth controlled by a shoot identity gene *Dt1*, the functional counterpart of the *Arabidopsis TFL1*, a floral suppressor gene primarily expressed in SAMs. Mutations in *TFL1* and *Dt1* result in the shoot apical meristem (SAM) switching from vegetative to reproductive state to initiate terminal flowering and thus show determinate stem growth. A second soybean gene (*Dt2*) regulating the stem growth habit was identified, which, in the presence of *Dt1*, produces semi-determinate plants with terminal racemes similar to those observed in determinate plants. Our recent work demonstrates that *Dt2* was a dominant MADS domain factor gene homologous to the *Arabidopsis* floral meristem (FM) identity gene *AP1*. However, unlike *AP1*, whose expression is limited to FMs in which the expression of *TFL1* is repressed, *Dt2* appears to repress the expression of *Dt1* in the SAMs to promote early conversion of the SAMs into reproductive inflorescences, resulting in semi-determinate stems. Given that *Dt2* is not among several soybean genes most closely related to *AP1*, as revealed by phylogenetic

analysis, and that semi-determinacy is rarely seen in wild soybeans, *Dt2* appears to be a recent gain-of-function mutation that has re-shaped the regulatory networks in the palaeopolyploid soybean.

17:20-17:40 Presenter: Julie Hofer (ICRISAT, Patancheru, India)

Sequence analysis of a pea (*Pisum sativum*) fast neutron mutant population identifies the gene corresponding to *Stipules reduced*

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A fast neutron mutant population developed in *Pisum sativum* (pea) for gene discovery has been used to identify eight morphology genes, two pigmentation genes and four seed metabolism genes to date. These previous studies showed that a range of deletion sizes exists in the population and that individual lines are expected to carry seven deletions, on average. The population is therefore suitable for both forward and reverse genetics, whereby high throughput sequence data is searched systematically for deleted sequences. As a test of the utility of the population for forward genetics, we screened Restriction Site Associated DNA (RAD) sequence data and identified a genetic determinant of stipule organ size, the *Stipules-reduced* (*St*) gene. A total of 994 sequence read tags were identified as absent from a *st/st* FN mutant line, compared to tags present with a read depth of at least 150 in isogenic *St/St* lines. Absent tags were used to identify 498 pea transcript contigs, which were filtered by BLAST against all sequence tags from the *st/st* line. This identified 43 candidate *St* transcripts, which were mapped in silico in Medicago. Genomic regions with clusters of adjacent-mapped candidates were investigated further using PCR assays and sequence analysis on mutant and wild type lines. A transcription factor on Chromosome 5 was identified as *St* and five pre-existing mutant alleles were characterized. This gene identification approach is readily applicable to any genome, even where extensive sequence is not available.

17:40-18:00 Presenter: Jocelyn Ozga (University of Alberta, Canada)

Role of gibberellins in seed coat development and photoassimilate partitioning in developing pea (*Pisum sativum* L.) seeds

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Gibberellins (GAs) are known to play a key role in early seed development. To understand the involvement of GAs as a sink strength determinant during rapid embryo growth and the early seed storage phase (8 to 20 days after anthesis [DAA]) of pea (*Pisum sativum* L.), seed coat and cotyledon development were histologically compared among the GA biosynthesis mutant lines *Ih-2* and *Ih-1* (mutant alleles of the LH gene that codes for the GAbiosynthesis enzyme ent-kaurene oxidase), and their wild-type line LH. *Ih-2* and *Ih-1* reduce GA1 levels (54% and 84%, respectively) in young pea seeds. The *Ih-2* mutation reduces seed size, but the *Ih-1* mutation only has a transient effect on seed size during development (Swain et al., 1993, Planta 191:482). In our study, we observed marked differences in celldifferentiation and expansion in the

seed coats of the *lh* mutants compared to the wild-type LH line. Development of the seed coat layers in *lh*-2 was affected to a greater extent than *lh*-1 when compared to LH. With respect to photoassimilate partitioning, the mobilization of starch from the seed coat to the embryo at the initiation of the storage phase in the embryo (14-16 DAA) was delayed by approximately 2 days in the *lh*-1 mutant, and greater than 4 days in the more severe *lh*-2 mutant. These data support that GAs regulate specific aspects of seed coat development, and as a result, can modify photoassimilate partitioning into the developing embryo.

IFLRC Applied Genetics and Genomics; Abiotic Stress Tolerance Chair: Ashutosh Sarker (ICARDA, India)

16:00-16:30 Presenter: Steve Beebe (CIAT, Cali, Colombia)

Breeding for tolerance to low soil phosphorus: the example of common bean

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Abiotic stress limits crop yields across the tropics. Poor soil fertility is widespread, especially low soil phosphorus (P) availability. The wild ancestor of common bean evolved in an environment in which abiotic stress was seldom intense, and thus the domesticated common bean is not inherently well adapted to poor soil conditions. In spite of this, there is sufficient genetic variability to improve adaptation, based on multiple tolerance mechanisms. Large seed size can supply greater nutrients and carbon for early plant growth when roots for nutrient uptake are still not well developed. Several root traits can contribute to better P acquisition from soil. Translocation of resources to grain is inhibited under stress and but is also subject to improvement. Crop phenology influences nutrient acquisition, and a longer growth cycle favors greater nutrient capture. Different mechanisms may be relatively more effective under different levels of stress, contributing to GxE. Interactions of drought with low P can result in more severe yield losses than with individual stresses. Experience with common bean suggests that under combined stress of low soil P and drought, addressing nutritional constraints may be the first line of defense, while some genotypes respond well in spite of multiple stresses.

16:30-16:45 Presenter: Shane Rothwell (Lancaster University, UK)

Liming (to recommended rates) limits legume growth and gas exchange by increasing root-toshoot signalling of the phytohormone abscisic acid

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Soil acidification is a natural process that can be hastened by intensive agricultural practices such as excessive use of mineral nitrogen fertilisers. If unchecked, these may negatively affect crop yield via altered nutrient availability and aluminium toxicity. Impacts of low soil pH are traditionally managed by applying lime (calcium carbonate) to target soil pH ranges (often 6-6.5) that optimise nutrient availability and subsequent yield. However, recommended rates of liming can decrease crop yields in susceptible soil types, possibly by reducing phosphorous availability though there is little mechanistic information on how plant physiological responses limit yield. Shoot dry weight of pot grown pea (*Pisum sativum* L. cv.

Alderman) amended with lime to target pH 6.5 (recommended rate) was reduced by 38% and gas exchange (stomatal conductance and photosynthesis) was inhibited by 50% and 32% respectively when compared to un-limed control plants (pH 5.7). Although lime increases rhizospheric calcium concentrations to levels significantly higher than that required for adequate crop nutrition, xylem calcium concentrations (a potential antitranspirant) were not increased (Rothwell & Dodd 2014; *Plant & Soil* in press). Instead, xylem sap and tissue analysis suggest that reduced gas exchange is caused by an increase in the plant hormone abscisic acid (ABA) which decreases stomatal conductance. The ABA deficient mutant pea '*wilty*' showed an attenuated stomatal response to liming, apparently confirming that increased ABA is mediating legume responses to low phosphorus availability under recommended rates of liming.

16:45-17:00 Presenter: Ralf Metzner (Institute of Plant Sciences, Jülich, Germany)

Monitoring roots, nodule and pod development in vivo: New perspectives on legume development

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Seed filling, root and nodule development are some of the key processes involved in abiotic stress resistance. However, these are complex processes hidden either by pod tissue (seed filling) or by soil (roots and nodules), so studying them guantitatively in-vivo is challenging. Non-invasive (3D) imaging techniques such as magnetic resonance imaging (MRI) can be used to investigate hidden structural development both above- and belowground. Co-registered positron emission tomography (PET) allows the acquisition of functional information by mapping of recent carbon investment of the plant and its dynamics e.g. in response to stress. For tasks not requiring spatial (volumetric) resolution, such as monitoring changes in pod water and dry-matter content, low field nuclear magnetic resonance relaxometry with portable devices (pNMR) can be applied. Such devices allow sensor-like applications in the greenhouse and the field. In the current contribution we will demonstrate the application of MRI to follow the development of root system architecture in soil grown pea and bean genotypes and monitor the progress of nodulation by repeated 3D mapping of nodule distribution. By correlating these maps with PET, monitoring carbon import, we get first insights into the functionality of nodules in the soil. Additionally we will demonstrate monitoring changes of bean pod water and dry-matter contents over the course of several weeks with pNMR. We discuss the potential and challenges of all three techniques (MRI, PET and pNMR) for application in legume research.

17:00-17:15 Presenter: Jens Berger (CSIRO Plant Industry)

Chickpea phenology and evolution

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Among broad-acre crops, chickpea has had a unique evolutionary trajectory through a series of bottlenecks that have selected for environment-specific phenological controls, adaptive strengths and weaknesses that still define where the crop is grown today. Originally a narrowly distributed, vernalization-responsive Mediterranean winter annual (*Cicer reticulatum*) from SE Anatolia, chickpea is now a widely distributed crop predominantly grown in warmer South Asian or spring-sown Mediterranean/temperate systems. The advent of Mediterranean spring-sowing in the Bronze Age and subsequent spread to

warmer climates in the south and southeast (South Asia) modified chickpea selection pressures: elevating the role of terminal drought and high temperature stress, and reducing the role of low winter temperatures. This led to the evolution of regionally-appropriate regulation of phenology through 3 distinct mechanisms:

The loss of the vernalization response, an unreliable regulator of flowering time in warmer, variable climates. An increase in temperature response as vegetative phase temperatures increased from north to south. Temperature-photoperiod compensation. Inverse relationships between temperature and photoperiod responses in Mediterranean germplasm provided the plasticity to facilitate expansion to warmer southern climes.

These mechanisms allow chickpea to match phenology with growing season length and avoid its principal stresses: the almost ubiquitous terminal drought, and winter cold, damaging at the vegetative phase largely in Mediterranean climates, but delaying pod set and exacerbating terminal drought almost universally. The move from north to south, and associated phenological changes have had wider ramifications for chickpea adaptation. While high temperature tolerance is comparatively common in chickpea, reproductive chilling tolerance is extremely rare, and more likely to reside in its Mediterranean winter annual wild relatives. For further chickpea improvement it is essential to understand the interplay between phenology and specific adaptation, so that we focus on adaptive traits that augment, rather than oppose the crop's principal strategy of stress avoidance.

17:15-17:30 Presenter: Shiv Kumar (ICARDA)

Extra short duration lentil for rice-based cropping systems in the Indo-Gangetic Plain of South Asia

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Lentil (*Lens culinaris* Medik. ssp. *culinaris*) has been an integral part of rice-based cropping system in the Indo-Gangetic plain (IGP) of South Asia, mainly because of its ability to thrive comparatively well under water-limiting environments. The expansion of productive boro-rice system, particularly in irrigated region of Bangladesh and northeastern India, has emerged as a threat to lentil cultivation in the absence of appropriate lentil varieties, which can fit in the system. The rice-rice system provides a short-season window of 100-110 days in which an extra short duration lentil variety (90-100 days) can successfully be grown. We have developed short duration breeding lines which can mature in 90-100 days in South Asia and are under testing in preliminary yield trials for their yield performance. Similar opportunity also exists for extra short duration lentil in the rainfed rice growing areas of South Asia dominated by medium to long duration varieties of rice and termed as rice fallows. The top soil layer generally dries completely at the time of the rice harvest and thus planting of a post-rainy season crop is not feasible. Under such conditions, extra-short duration lentil lines can convert these mono-cropped areas into double cropped areas and thus increase lentil production and sustain productivity of the rice-based system. Increased adaptability of these lines to marginal soil conditions will help increase the overall cropping intensity, system productivity, sustainability and profitability.

17:30-17:45 Presenter: Imran Malik (University of Western Australia)

Variation in physiological responses in pea and lentil to soil waterlogging

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Pea (Pisum sativum L.) and lentil (Lens culinaris subsp. culinaris Medik) are grown under rainfed conditions after rice in South Asia. However, production of these legumes in rice-based cropping systems is difficult due to their susceptibility to waterlogging. A pea and a lentil cultivar from Australia and a germplasm accession from each of Bangladesh and Pakistan were evaluated for waterlogging tolerance in glasshouse and in laboratory experiments. In the laboratory, O2-consumption rates at germination were measured under aerated conditions or after 72 h under water. O2-consumption rate differed significantly among genotypes and increased up to 6-fold after 72 h under water. Waterlogging tolerance at early developmental stage and their recovery was evaluated in pot soil system. With increasing severity of waterlogging treatment germination rate decreased; in waterlogged-10 (water level 10 mm below soil surface) germination rates were 100 % for peas; however, it was 75% for lentils. In waterlogged+10 (water level 10 mm above soil surface), at the end of the experimental period (14 d treatment) all seeds of Australian pea cultivar were moulded and the germination rate of the other genotypes was reduced to 50 -66%. Root length and root porosity differed significantly between pea and lentil genotypes during waterlogging, and differences in tolerance to waterlogging and recovery during the vegetative stage were also demonstrated. These observations of marked differences in waterlogging tolerance within a restricted sample of pea and lentil germplasm from different origins indicate that selection within a broader germplasm sample is clearly warranted.

17:45-18:00 Presenter: Hari D. Upadhyaya (ICRISAT, India)

Capturing Variations for Multiple Stress and Agronomic Traits for Improvement of Grain Legumes

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Grain legumes are the primary sources of oil, fiber, and protein-rich food and feed. Crops adaptation to climate change is one of the major challenges to scientific community in the 21st century. Grain legumes have narrow genetic base due to bottlenecks associated with domestication. Genes for desirable traits are embedded in biodiversity. ICRISAT genebank holds 49,819 accessions of its mandate grain legumes. Core/mini core collections have been suggested as resource to discover new sources of variations for enhanced use of germplasm in crop breeding. Using mini core approach trait-specific genetically diverse germplasm for resistance to drought, salinity, water logging, heat stress, pest, diseases, herbicide, and for agronomic and nutritional traits have been identified. The evidence to date suggests increased use of germplasm in legumes breeding at ICRISAT since formation of mini core collections. The crops have moved from orphan to genomic resources rich crops. The chickpea and pigeonpea genomes have been decoded. Resistance to some pest and diseases has been successfully transferred from wild relatives to

cultivated species. Synthetics in groundnut are recycled for capturing diversity lost during domestication. Increased use of agrobiodiversity together with modern genomics tools is crucial to coping with new challenges to agricultural production. Germplasm and breeding lines with unique traits are being sequenced, and comparison of sequence information of these unique germplasm with reference genomes will enable us to detect variation at nucleotide level. This will provide researchers opportunity to relate these differences with beneficial traits for enhancing trait value in breeding programs.

Tuesday, July 8, 2014

Seeds and Nutrition

ICLGG Seeds and Nutrition

Chair: Georgina Hernandez (Universidad Nacional Autónoma de México, Mexico)

11:00-11:30 Presenter: Jerome Verdier (Shanghai Institutes of Biological Sciences, CAS, China)

Deciphering regulation of legume seed nutritional composition

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Legume seeds are a primary source of proteins, lipids, carbohydrates, secondary metabolites, and minerals. Few regulatory genes have been discovered that control legume seed storage metabolism. Therefore, we have initiated a systems biology approach to decipher seed storage metabolism in the model legume, *Medicago truncatula*. To unravel these mechanisms, we have been using different approaches to model the seed development. An *in silico* gene regulatory network during seed development was built, which linked transcription factor genes to their potential target genes, enzymatic genes to metabolites and proteins, hormones to signaling genes and small RNA to transcription factors. In parallel, a genome-wide association study using resources developed by the *Medicago* HapMap project has been initiated and should reveal sequence polymorphisms associated with distinct seed composition phenotypes such as carbohydrate, protein, lipid, secondary metabolite and mineral contents. Finally, a comparative genomics study between legumes with contrasting seed storage composition has been conducted to highlight common and different mechanisms within legume seed development. The combination of these approaches will lead to the discovery of genes controlling seed nutritional composition and seed quality in legumes. In a near future, it should lead to new ways to improve the nutritional quality of legume seeds.

11:30-11:50 Presenter: Claire Domoney (John Innes Centre, Norwich, UK)

The wild side of seed quality improvement: discovering novel genetic variation that impacts on the control of visual traits and composition in *Pisum sativum* L. (pea)

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Germplasm resources of *Pisum*, including natural variants, EMS and fast neutron mutagenised populations, harbor novel variants of genes that impact on seed metabolism, nutritional quality and additional traits of economic importance. Our studies of variation within genes that control chlorophyll metabolism during late stages of seed maturation show the considerable allelic variation that exists outside standard breeding genetic resources. The role of retro-elements in shaping the diversity of variation for this biochemical process will be discussed, alongside the potential of novel genetic variants to stabilize desirable seed visual traits. Mutations in genes involved in the control of seed composition enable the profile of seed constituents to be altered dramatically. Allelic variants of genes encoding starch-branching enzyme, ADP-glucose pyrophosphorylase and other genes involved in sucrose metabolism lead to different contents of starch and profiles of sugars within seeds. Mutations in genes encoding albumin 2, trypsin inhibitor and lectin have been identified and combinations of null mutations for these offer prospects for increasing the yield of digestible protein. The high-throughput screening and characterization of *Pisum* resources will assist greatly with the adoption and selection of superior alleles of genes relevant to the breeding of legume food and feed crops for improved yield and quality.

11:50-12:10 Presenter: Mitch Lucas (University of California Riverside, USA)

Using SNPs to Breed Cowpeas with Large Seeds

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Seed size distinguishes most crops from their wild relatives and is an important quality trait for the grain legume cowpea. In order to breed cowpea varieties with larger seeds we associated SNP markers from a 1,536-plex GoldenGate assay with seed size by studying 7 RIL populations, a subset of the USDA core collection, and synteny to the related legume soybean. These QTL discovery and characterization efforts described SNP haplotypes and their predicted effects in different pedigrees such that marker-assisted breeding schemes could be designed for cowpea with larger or smaller seeds. Parents for crosses were chosen based on genotype calls with a goal of pyramiding large seed haplotypes into different seed types. Foreground and background selection were performed during two cycles of backcrossing based on the KASP genotype calls of a genome-wide set of polymorphic markers. Progeny with very large seeds (up to 35g/100 seed) were developed through introgression of one QTL from IT82E-18 (18.5g/100 seed) into CB27 (22g/100 seed). Field testing for performance and enabling partner institutions for the deployment of markers is a continuing effort that will be enhanced later this year by the availability of a 60,000 SNP iSelect genotyping platform. This will be a core resource for collaboration with partners from Burkina Faso, Ghana, Nigeria, and Senegal. Coupling high-density genotyping with knowledge of physical and genetic maps provides excellent opportunities to identify genetic determinants underlying traits relevant to breeding objectives.

Biotic Stress

12:10-12:30 Presenter: Vijai Bhadauria (University of Saskatchewan, Canada)

Regulation of hemibiotrophy in the lentil anthracnose pathogen Glomerella truncata

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Anthracnose caused by *Glomerella truncata* is one of the most devastating fungal diseases on lentil. This pathogen exploits a hemibiotrophic infection strategy to colonize lentil plants, and the lifestyle transition from biotrophy to necrotrophy (the hemibiotrophic switch) is critical in anthracnose development. Mining of G. truncata-infected lentil transcriptome coupled with quantitative real-time PCR and Northern blot analysis identified a hemibiotrophic switch-specific gene CtNUDIX (Colletotrichum truncatum NUcleoside DIphosphate linked to moiety X). Nudix hydrolases are known to hydrolyze inositol pyrophosphates (IPs), and some IPs are involved in endocytotic trafficking in plants. Western blot analysis and in planta expression assays revealed that CtNUDIX is an effector protein and secreted in lentil cells to induce cell death. Green fluorescent protein tagged CtNUDIX was localized in endocytotic vesicles located at the plasma membrane, suggesting that CtNUDIX perturbs the plant cell surface dynamics to trigger a hypersensitive cell death like response. Furthermore, overexpression of CtNUDIX in G. truncata and heterologous expression in the model pathogen *Magnaporthe oryzae* blocked the hemibiotrophic switch and resulted in incompatibility with hosts lentil and barley, respectively. A hypersensitive cell death response was also observed in infected host cells associated with the biotrophic invasive hyphae. Taken together, these results provide compelling evidence that G. truncata secretes CtNUDIX in lentil cells to shut down the biotrophic phase by inducing cell death and to initiate the destructive necrotrophic phase resulting in anthracnose lesions. CtNUDIX can therefore serve as an excellent target to control diseases caused by hemibiotrophic fungal pathogens.

IFLRC Seeds and Nutrition

Chair: Albert Vandenberg (University of Saskatchewan, Canada)

11:00-11:30 Presenter: Francesca Sparvoli (IBBA, CNR, Milan, Italy)

Iron biofortification in common bean

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Iron deficiency anaemia is the most prevalent micronutrient deficiency affecting more than 2 billion people throughout of the world. Iron bioavailability may be strongly reduced by the presence in the seeds of iron absorption inhibitors, such as phytic acid (IP6) and polyphenols (PP). In different crop species low PP

genotypes can be easily identified, conversely natural variability in IP6 content is not very high and the best way to gain significant IP6 reduction is by obtaining low phytic acid (*Ipa*) mutants. Although such type of mutants have been identified in several grain crops, often IP6 reduction is associated with negative agronomic traits, such as lower seed viability and emergence, reduced plant growth rate and grain yield. These findings may limit the use of *Ipa* mutants for iron biofortification, since acceptable agronomic performance should be guaranteed to small farmers and poor populations that would benefit from biofortified crops.

Due to its worldwide consumption and nutritional qualities, common bean has been identified as a strategic target crop for iron biofortification. We have isolated a bean *lpa* mutant having a 90% seed IP6 reduction and showed it is defective in a MRP type ATP-binding cassette transporter (Pvmrp1) specific for IP6. Agronomic analyses of the original *lpa* mutant and of derived *lpa* lines have shown that seedling emergence, seed yield and plant growth were not affected. *In vitro* and *in vivo* analyses confirmed significant increases of iron bioavailability in bean *lpa* seeds.

11:30-11:45 Presenter: William Erskine (University of Western Australia)

Selenium biofortification of lentil in Australia and Bangladesh

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A low concentration of micronutrients in the diet causes micronutrient malnutrition. Biofortification by agronomic management or plant breeding approaches can reduce such malnutrition in rural areas of developing countries. Globally over one billion people suffer from selenium (Se) deficiency. Lentil can be an effective food to supply dietary Se to affected populations. This study used a baseline survey in farmers' lentil fields in Bangladesh and conducted field experiments in Bangladesh and Australia to unravel genotypic and environmental effects on seed Se concentration and to evaluate foliar Se application.

A farmers' field survey and evaluation of seven lentil genotypes at four locations were conducted in Bangladesh in 2010/11. Soil and lentil seed Se concentration in farmers' fields averaged 163 and 312 µg/kg, respectively. There were significant genotype and location differences for seed Se and seed yield. But the genotype-location interaction was non-significant for seed Se concentration.

In Australia trials on Se foliar application at two locations and an evaluation of 12 genotypes at seven locations were undertaken in 2011. Foliar application of 40 g/ha of Se as K2SeO4 increased seed Se concentration from 200 to 2772 µg/kg, but did not affect seed size or yield. The genotypic evaluation, as in Bangladesh, showed significant genotype and location effects for seed Se, but the interaction was non-significant.

In summary, foliar Se application is an efficient approach to improve lentil seed Se concentration. With clear genetic differences in Se uptake in lentil exhibited, there is scope to breed lentil for an improved Se

seed content.

11:45-12:00 Presenter: Peter Zahradka (Canadian Centre for Agri-Food Research in Health and Medicine)

The structural properties of arteries are altered by consumption of pulses: Implications for treatment of atherosclerotic disease

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A human study was conducted to evaluate the effects of eating 1/2 cup per day of a mixture of pulses (beans, peas, chickpeas, lentils) over an 8 week period on vascular function in persons with peripheral artery disease (PAD), a manifestation of atherosclerosis in which blood flow to the legs is reduced. A cohort of 26 men and women was provided with pulse-rich foods (14 different items to provide variety) to incorporate into their usual diet. This study revealed that consuming pulses above the normal amounts usually eaten by Canadians could improve the ankle-brachial index, the primary tool for diagnosis of PAD. by increasing blood flow to the legs. No pharmaceutical drug currently available exhibits this capability. Furthermore, these results suggest that dietary pulses affect the physical properties of blood vessels. We therefore conducted an animal study to investigate the effects of individual pulses on arterial structure and function. Spontaneously hypertensive rats were randomly assigned (n=8/group) to diets containing 30% (wt/wt) cooked and freeze-dried beans, peas, lentils, chickpeas, a pulse mixture or no pulses for 4 weeks. Blood pressure (BP) and pulse wave velocity were measured weekly, and morphometry was performed at termination. Arterial stiffness assessed by pulse wave velocity was unchanged, however, lentils decreased BP and reduced the aortic media: lumen ratio. Our results are the first to show that eating a specific food can reverse the main cause of atherosclerotic disease, and that the amount of pulses providing this benefit can be reasonably incorporated into our regular diet.

12:00-12:15 Presenter: Laura McBreairty (University of Saskatchewan)

A pulse-based diet and exercise training in women with polycystic ovarian syndrome: Effects on body composition, blood lipids and reproductive measures

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Polycystic ovarian syndrome (PCOS) is an endocrine disorder that predisposes women to increased risk of heart disease, diabetes, infertility and endometrial cancer with annual cost of \$4 billion in the United States. We hypothesized a pulse-based diet (e.g. beans, lentils) would have a positive effect on body composition, reproductive measures and serum lipid profiles. Thirty-one women with PCOS aged 18-35y with a mean body mass index of 33 were randomly assigned to groups receiving a pulse-based diet (n=17) or the National Cholesterol Education Program (NCEP) therapeutic lifestyle changes (TLC) diet (n=14) for 16 wks while participating in an exercise program. During the intervention, both groups lost body mass (p<0.001; Pulse -3.6 vs TLC -3.0 kg), percent fat mass (p=0.0013; Pulse -1.0 vs TLC -1.5 %),

trunk fat mass (p=0.0005 Pulse -1.0 vs TLC -1.5 kg) as well as lean body mass (p<0.02; Pulse -1.3 vs TLC -0.4 kg). Both dietary interventions also resulted in more women exhibiting regular menstrual patterns (p<0.01) and a decreased antral follicle count in the right ovary (p=0.04); however, only the pulse diet reduced total cholesterol to HDL ratio (4.1 to 3.7 p<0.005). The pulse-based diet and TLC diet reduced body fat, and improved reproductive endpoints; however, the pulse-based diet appeared to provide additional benefits by improving lipid profile. Diet and exercise are important in alleviating the personal health and economic costs associated with PCOS.

Supported by Agriculture and Agri-Food Canada and the Saskatchewan Pulse Growers

12:15-12:30 Presenter: Susan Arntfield (University of Manitoba, Canada)

Changes in levels of antioxidant and selected antinutritional factors in peas and beans due to the application of heat

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Increased consumption of legumes offers many nutritional advantages including high levels of antioxidants but antinutritional compounds such as phytic acid and trypsin inhibitors can limit legume consumption. This work represents an overview of changes in antioxidants based on a DPPH analysis (AOX) and total phenolics (TP) as well as the antinutritional factors phytic acid (PA) and trypsin inhibitors (TI) due to micronization (infrared heat) and roasting (convection heat). Changes in whole seeds as well as changes during the preparation of noodles, flat breads and extruded snack foods have been investigated. Both infrared and convection heating reduced PA (40-60%) and TI (88-100%) in whole seeds with greater reductions with roasting. For beans, there were also decreases in TP (up to 25%) and AOX (up to 55%), but for peas both TP and AOX increased. When these materials (peas specifically) were dehulled and milled prior to use, the effects of heat were greatly reduced. Noodles prepared from flour containing navy bean and yellow pea had reduced levels of PA and TP compared to the raw blend, but TIs were unchanged. When preparing tortillas from flours that included varying levels of peas and beans, PA and TI were again reduced but TP increased by 40-75%; however AOX activity decreased slightly. The high temperatures used in extrusion eliminated TI and reduced all other parameters. The temperature and type of heating influence both antinutritional and antioxidant activity; however conditions can found to reduce antinutritional factors while minimizing loss of antioxidants.

ICLGG Biotic Stress

Chair: Scott Jackson (Univ. of Georgia, USA)

14:00-14:30 Presenter: Kiran Mysore (The Samuel Roberts Noble Foundation, USA)

Identification of novel sources of resistance in a nonhost plant, *Medicago truncatula*, against Asian Soybean Rust caused by *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 against ASR will provide an avenue to engineer soybean for durable resistance against ASR. We found that *Medicago truncatula*, a model legume, conferred nonhost resistance against ASR. Tobacco retrotransposon, *Tnt1*, has been used to mutagenize and tag the whole genome of *M. truncatula*. A forward genetics approach using *Tnt1* tagged *M. truncatula* lines has been established to identify genes that confer nonhost resistance to *P. pachyrhizi*. Several *M. truncatula Tnt1* mutants with altered response to *P. pachyrhizi* have been identified and being characterized. *irg1* (inhibitor of rust germ-tube differentation1) mutant inhibited pre-infection structure differentiation of *P. pachyrhizi* and several other biotrophic pathogens. *IRG1* encodes a Cys(2)His(2) zinc finger transcription factor, PALM1 that also controls dissected leaf morphology in *M. truncatula*. Characterization of other mutants will also be presented.

14:30-15:00 Presenter: Nadine Ilk (The Sainsbury Laboratory, Norwich, UK)

Translational research at The Sainsbury Laboratory- from the laboratory to the farm-

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The coming century poses some formidable challenges for agriculture. We must increase food and feed production from the same amount of land 50% by 2030, against a backdrop of climate change, a reduction in water availability and rising energy costs. In addition, we want to accomplish these goals with respect for the environment, a reduction of chemical pesticides and increased food safety. Plant diseases cause severe crop losses worldwide with devastating effects on food and feed production. Over the past decades the understanding of how plant pathogens cause disease and how plants defend themselves against them has been refined. Within TSL we not only aim to further this understanding through fundamental research, but we also aim to improve current methods of disease control via translational research. The Soybean Rust research group within TSL performs such translational research in collaboration with the 2Blades foundation, the Universidade Federal Viçosa and our commercial partner DuPont-Pioneer. The strong combination of expertise of team members and collaborators in various aspects of host-pathogen interactions enables us to make disproportionate gains in speed of discovery and deployment. Using this know-how, we are making inroads in understanding the basic biology of the causal agent of Asian Soybean Rust, the obligate biotrophic fungus *Phakopsora pachyrhizi*, and into the development of plant disease resistance against this pathogen.

15:00-15:20 Presenter: Marie-Laure Pilet-Nayel (INRA, Rennes, France)

Translational genomics for resistance to *Aphanomyces euteiches* between *Medicago truncatula* and pea

Pilet-Nayel, M-L.^{1*}, Bonhomme, M.², André, O.², Hajri, A.¹, Boutet, G.¹, Badis, Y.², Chantret, N.³, Ronfort, J.³, Young, N.D.⁴, Baranger, A.¹, and Jacquet, C.². ¹INRA, UMR IGEPP, Le Rheu F-35653, France; ²UPS-CNRS, LRSV, F-31326 Castanet-Tolosan, France; ³INRA, UMR AGAP, Montpellier F-34060, France; ⁴University of Minnesota, Saint Paul, MN 55108, USA. *(Marie-Laure.Pilet@rennes.inra.fr)

Common root rot, due to Aphanomyces euteiches, is a very damaging disease of legumes, especially pea,

in many countries. Several consistent quantitative trait loci controlling partial resistance were identified in different pea genetic pools. Genomic basis of resistance to *A. euteiches* was investigated in the model legume *Medicago truncatula* in order to identify candidate genes underlying Aphanomyces resistance loci and analyze synteny of resistance loci between pea and *M. truncatula*. High density genome-wide association mapping was conducted in a collection of 179 *M. truncatula* accessions, for resistance to five reference *A. euteiches* isolates of main pea and alfalfa pathotypes. Two main genomic regions carrying candidate SNPs were associated with resistance. The first one corresponded to an F-box protein encoding gene at the *prAe1/AER1* loci on chromosome 3 and the second one to a NBS-LRR resistance protein encoding gene underlying a new resistance locus on chromosome 4. The candidate genomic regions identified in *M. truncatula* genome. The F-box gene syntenic region co-located with the minor QTL *Ae-Ps3.2* on pea LGIII and the NBS-LRR gene syntenic region was positioned about 50 cM far from the major QTL *Ae-Ps7.6* on pea LGVII. Work is in progress to precise synteny between Aphanomyces resistance loci in pea and *M. truncatula* and to search for pea genes orthologous to the *M. truncatula* candidate genes identified.

15:20-15:40 Presenter: Zenglu Li (University of Georgia, USA)

An Integrated Molecular Breeding Approach for Breeding Nematode Resistance in Soybean

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Soybean nematodes including soybean cyst nematode (SCN), root-knot nematode (RKN) are one of the most destructive pests in soybean production in the world. These nematodes are able to live in the soilfor years and cause a significant loss of soybean yield. Phenotyping for nematode resistance is laborintensive and time-consuming. Development of nematode resistance soybean cultivars is the most effective way to controlling nematodes in soybean production. PI 96354 was identified having a high level of resistance to RKN galling and egg production. Two QTLs were found to condition the resistance in PI 96354, a major and minor QTLon chromosome 10 and 8, respectively. Using the recombinants derived from the cross of PI 96354 x Bossier as well as soybean reference genome sequence, we have identified four candidate genes with cell-wall modification function responsible for RKN resistance. These candidate genes have several mutations in promoter, exon, 5', and 3'UTR regions, which were confirmed with sequencing and RT-PCR. Two mutant SNPs were selected to develop KASP assays to detect the resistant alleles. Based on the SNP assays, we have developed a high-throughput procedure for seed and leaf samples to select the progeny for RKN resistance in our breeding program. Two genes, *Rhg1* and Rhg4 have been widely used for breeding SCN resistance. Based on the published sequences, we have also developed SNP assays to select for SCN resistance. The genotyping and selection workflow will be presented.

IFLRCSeeds and NutritionChair:Khalid Daoui (Centre Régional de la Recherche Agronomique de Mèknes, Morocco)

14:00-14:30 Presenter: C.L. Laxmipathi Gowda (ICRISAT, India)

Impact-Oriented Legume Seed Systems in developing countries of Sub-Saharan Africa and Asia

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Seed is the most important input for increasing crop productivity globally, especially for the smallholder farmers of Sub-Saharan Africa and Asia. Timely availability of quality seeds of improved crop varieties even at remote locations, at affordable prices, and required quantity, is essential for smallholder farmers to adopt improved varieties. During the past two decades several high yielding varieties of different legume crops have been developed, but only a few have been widely adopted. One of the main reasons for slow rate of adoption of these varieties is due to inadequacy of the seed production and delivery systems. Many international research centers, national systems, donor agencies, and development projects have been working together to strengthen the seed systems in the national programs, and also regionally. There are many bottlenecks in legumes seed systems due to (i) low multiplication ratio, (ii) bulkiness and reduced storability of seeds, (iii) fragmented seed demand (agro-ecology/market based) and (iv) inadequacy of national policy support, coupled with limited interest by the private sector. Basically, two types of seed systems operate: (i) formal seed system (supplying 5%) - organised sector with production, processing and delivery of quality seeds (ii) informal system (supplying 95%) - less organised but working reasonably in developing countries of Asia and Africa. Several successful models have been studied in different countries to strengthen both seed systems, but it is clear that each system needs to be fine-tuned to suit local situations.

14:30-14:45 Presenter: Felix Dapare Dakora (Tshwane University of Technology, South Africa)

Role of symbiotic cowpea (*Vigna unguiculata L. Walp.*) as a major food legume for nutritional security in Africa

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Cowpea (*Vigna unguiculata* L. Walp.) is the most important grain legume in Africa. It meets the food and nutritional security of about 1.1 billion people in the African continent. Studies of its grain yield and food quality are therefore crucial for the human nutrition and health of many Africans. The leaves and grain of cowpea are consumed in large quantities in Africa and elsewhere in the world. The grain yield of high performing cowpea varieties can be as high as 2000-3000 kg.ha-1 in South Africa, and 2500 kg.ha-1 in Ghana. Protein levels in the edible leaves are high and range from 29 to 40%, while grain protein also ranges from 23 to 40% depending on the variety and its symbiotic functioning. In fact, the grain protein of farmer-selected cowpea varieties such as Soronko and Bengpla can be as high as 40%, a level comparable to that of soybean. Both leaves and grain also contain very high concentrations/amounts of macronutrients and trace elements, the latter needed for overcoming micronutrient deficiency in rural Africa. Evidence from field and glasshouse studies has shown that, as with leaf and grain protein, the accumulation of mineral nutrients by cowpea is symbiosis-dependent. In the field, cowpea varieties with high N2-fixing ability accumulated more nutrient elements than low fixers, while in nodulation assays,

rhizobia with high N2-fixing efficiency generally elicited greater mineral accumulation in cowpea shoots when compared to strains with low symbiotic efficacy. These findings on the symbiosis-induced mineral accumulation in cowpea offer an additional explanation for the benefits of legume/cereal rotations beyond the known N contribution by the legume, and further validate cowpea as an important food legume for nutritional security in Africa.

14:45-15:00 Presenter: Ramakrishnan Nair (AVRDC – The World Vegetable Center South Asia, India)

Enhancing production and consumption of mungbean

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Mungbean is a short duration (55-70 days) grain legume that fits well into the cereal cropping systems prevalent in the tropics and is currently being grown on more than 6 million hectares worldwide. Mungbean is consumed by boiling whole dried seeds or split seeds, as *dhal* (porridge), bean sprouts or bean paste, or processed into high-value noodles or confectionery. Owing to its palatable taste and nutritional guality, mungbean has been used as an iron-rich whole food source for baby food. Broadening the genetic base of mungbean is a priority and breeding programs increasingly use crosses between unrelated parents and interspecific hybridization. This is important to tackle problems such as mungbean yellow mosaic disease, for which currently available sources of resistance are insufficient to cope with disease pressure in certain locations. Resistance to Cercospora leaf spot, powdery mildew and bruchids are other traits of importance to improve productivity. Among abiotic stresses, tolerance to heat stress and salinity are desirable. There is scope for improvement in starch, iron and zinc content and protein guality through plant breeding, which will lead to further enhancement of the nutritional value of the crop. Active promotion of the nutritional value of mungbean with its proven agronomic advantages will support the case for further expansion in Asia and other parts of the world, including sub-Saharan Africa. The availability and accessibility of new mungbean cultivars with improved nutrient content and higher productivity will also help to reduce malnutrition, especially among women and children in developing countries.

15:00-15:15 Presenter: Jenny Wood (NSW Department of Primary Industries, Australia)

Imaging of grain legume seeds: understanding what is where and the influence on nutritive value, health benefits and quality traits

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Opportunities exist to add value to our grain legume crops through enhancing our understanding of their nutritive value, health benefits and other quality traits. It is also important to consider how these factors interact. Whilst there is a large amount of information available on the nutrient composition of the raw seeds, there is less detail available about the anatomical location of these nutrients and how these can affect quality traits like splitting and cooking.

Australian faba beans and chickpeas were investigated microscopically with preferential staining to highlight specific chemical components of the morphological structures within grain legume seeds. Differences were observed between varieties which help explain differences in nutritive value, ease of splitting, speed of cooking and potential benefits to human health.

This presentation will summarise the significant findings, dispel some industry myths and explain the relevance for breeding programs, grain legume processors, animal nutritionists, dieticians and consumers.

15:15-15:30 Presenter: Arun Shunmugam (University of Saskatchewan)

Biochemical and molecular characterization of low phytate pea lines

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Seeds of low phytic acid (*Ipa*) pea lines are low in phytic acid (IP6) and high in inorganic phosphorus (Pi). The present study was aimed at biochemical and molecular characterization of two lpa pea lines, 1-150-81, 1-2347-144, and their progenitor CDC Bronco. The Ipa lines did not significantly differ from CDC Bronco in the agronomic traits assessed except for lower grain yield. No inositol polyphosphates (IP1, IP3, IP4, IP5) other than phytic acid were detected in cotyledons of CDC Bronco and the Ipa lines at different seed developmental stages analyzed through HPLC. Total phosphorus concentration was similar in Ipa lines and CDC Bronco throughout seed development. To understand the genetic basis of the *lpa* mutation in pea, a 1530 bp open reading frame of myo-inositol phosphate synthase (MIPS) (EC 5.5.1.4) was amplified from CDC Bronco and the *lpa* lines. Sequencing results showed no difference in coding sequence in MIPS between CDC Bronco and Ipa lines. Transcription levels of both MIPS and myo-inositol pentakisphosphate 2-kinase (IPK1) were relatively lower at 49 DAF than at 14 DAF for CDC Bronco and Ipa lines. Recombinant inbred lines (RILs) developed from a cross between 1-2347-144 and CDC Meadow were evaluated in replicated field trials in Saskatchewan in 2011, 2012 and 2013. The RILs were genotyped using 1536 Single nucleotide polymorphic (SNP) markers in Illumina GoldenGate array and phenotyped using colorimetric assays. SNP marker associated with phytic acid phosphorus concentration was identified and mapped.

Wednesday, July 9, 2014

Nitrogen Fixation, Plant Nutrition and Legume Mega Project

ICLGG Legume Mega Projects

Chair: Jens Stougaard (Aarhus University, Denmark)

11:00-11:30 Presenter: Tim Close (University of California Riverside, USA)

Innovation Lab on Climate Resilient Cowpea

Close, T.J.^{1*}, Roberts, P.A.¹, Lonardi, S.¹, Cisse, N.², Drabo, I.³, Tignegre de la Salle, JB.³, Atokple, I.⁴, Kusi, F.⁴, and Boukar, Ousmane.⁵. ¹University of California, Riverside, California, USA; ²Institut Senegalais Recherches Agricole (ISRA), Senegal; ³Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso; ⁴Council for Scientific & Industrial Research - Savanna Agricultural Research Institute (CSIR-SARI), Ghana; ⁵International Institute of Tropical Agriculture (IITA), Nigeria. *(timothy.close@ucr.edu)

This five-year project, "USAID Feed the Future Innovation Lab for Climate Resilient Cowpea" began September 2013. It is under the umbrella of the Feed the Future Global Hunger and Food Security Research Strategy: Climate Resilience, Nutrition, and Policy in the Program Area "High-Yielding, Climate Resilient Legumes". The focal theme is breeding and genetics for cowpea, with the aim of increasing the yield potential and providing solutions for major production constraints. The project involves partnership between the University of California at Riverside and the leading cowpea breeders in four West African nations (Burkina Faso, Ghana, Nigeria, Senegal) in the Sudano-Sahelian region, which is the main production zone for cowpea. The areas of emphasis for variety improvement are resistance to the droughtpredisposed fungal pathogen Macrophomina phaseolina, drought tolerance at critical stages (early seedling, flowering, terminal) and heat tolerance during reproductive development. There are three basic objectives: 1) Foundation Development, 2) Training and 3) Implementation. Foundation Development includes the improvement of genotyping capabilities. In year 1 this includes the development of a 60,000 BeadAssay iSelect for SNP genotyping and its implementation to produce a higher density genetic map and baseline genetic knowledge about the breeding materials in each West African program. Drought tolerance of these same materials will be assessed in year 1 in sandbox assays to provide new baseline phenotypic data, supporting genotype-phenotype associations. A MAGIC population and diverse germplasm are also elements of this project. Training and implementation activities will occur through the life of the project.

11:30-12:00 Presenter: Doug Cook (University of California Davis, USA)

Taking a walk on the wild side: prospecting for climate resilience and nitrogen fixation traits in the wild progenitors of cultivated chickpea

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Crops are impacted in unintended, often negative ways during their domestication and breeding. Loss of adaptive alleles, fixation of deleterious alleles, and low genetic diversity in cultivated species constrains our ability to expand their cultivation into more extreme climates, marginal soils, or to situations with reduced agricultural inputs. We are addressing this need in chickpea, the world's second most important pulse legume, by harnessing the capacity of wild relatives to survive in harsh environments. Effective use

of wild germplasm in chickpea improvement requires new and systematic surveys of genotypes from natural environments, identification of adaptive alleles to environmental extremes, and incorporation of the diversity of wild alleles into purpose driven populations for trait analysis and breeding. We focus on climate resilience, nitrogen fixation and seed nutrient density, with the goal of more sustainable and stable production systems. We combine upstream ecology and genomics to assemble and characterize wild germplam; population development to remove barriers to use of wild alleles for trait assessment and breeding; and phenotyping and modeling of trait-gene associations to enhance the precision and rate with which wild alleles are applied to crop improvement. To date we have completed ecological characterization and genetic resource collection for ~1,100 novel accessions, used genotyping by sequencing and bioinformatics to deduce population genetic parameters, initiated whole genome resequencing, collected co-occurring bacterial symbionts, surveyed seed and flowering phenotypes, and initiated development of nested association mapping panels. We will present these results along with our vision for restructuring cultivated germplasm with wild alleles.

12:00-12:30 Presenter: Suk-Ha Lee (Seoul National University, Korea)

Genome Sequence of Mungbean and Vigna Speciation

Suk-Ha Lee

Department of Plant Science and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Mungbean (*Vigna radiata*) is a fast-growing, warm-season legume crop that is primarily cultivated in developing countries of Asia. We constructed a draft genome sequence of mungbean to facilitate genome research into the subgenus *Ceratotropis* and to enable a better understanding of the evolution of leguminous species. The draft genome sequence covers 80% of the estimated genome, of which 50.1% consists of repetitive sequences. In total, 22,427 high confidence protein-coding genes were predicted. Based on the *de novo* assembly of additional wild mungbean species, the divergence of what was eventually domesticated and the sampled wild mungbean species appears to have predated domestication. Moreover, the *de novo* assembly of a tetraploid *Vigna* species (*Vigna reflexo-pilosa* var. *glabra*) provided genomic evidence of a recent allopolyploid event. To further study speciation, we compared *de novo* RNA-seq assemblies of 22 accessions of 18 *Vigna* species and protein sets of *Glycine max* and *Cajanus cajan*. The species tree was constructed by a Bayesian Markov chain Monte Carlo method using highly confident orthologs shared by all 24 accessions. The present assembly of *V. radiata* var. *radiata* will facilitate genome research and accelerate molecular breeding of the subgenus *Ceratotropis*.

IFLRC N Fixation and Plant Nutrition

Chair: C.L. Laxmipathi Gowda (ICRISAT, India))

11:00-11:30 Presenter: Endalkachew Wolde-meskel (Hawassa University, Ethiopia)

Exploiting indigenous rhizobial biodiversity resources and symbiotic N2-fixation to benefit smallholder farmers: the case of Ethiopia Welde-meskel, E.^{1,2*}, Degefu, T.³, Frostegåard, A.⁴, Tena, W.², and Walley, F.⁵. ¹N2Africa Project-Ethiopia, International Livestock Research Institute (ILRI), P.O.Box 5689, Addis Ababa, Ethiopia; ²Hawassa University, School of Plant and Horticultural Sciences, P.O. Box 5, Hawassa, Ethiopia; ³Hawassa University, Department of Applied Biology, P.O. Box 5, Hawassa, Ethiopia; ⁴Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, Ås, Norway; ⁵University of Saskatchewan, Department of Soil Science, 51 Campus Drive, Saskatoon, S7N 5A8, Saskatchewan, Canada. *(E.Woldemeskel@cgiar.org)

Rhizobia are key resources as they strongly contribute to the nitrogen budget in many agricultural systems due to their nitrogen fixing capacity. Through North-South collaborative projects during last decade, we have isolated a large number of rhizobia (>500 strains) from different legume species growing in diverse agroecological zones in Ethiopia. Phylogenetic analyses revealed that the bulk of the Ethiopian strains are distinct from the hitherto known taxa of the family Rhizobiaceae. Recently, several new genospecies within this collection have been reported, including seven novel lineages within Ensifer, four within Rhizobium and three new species within Mesorhizobium (M. hawassense, M. shonense, M. abyssinicae). Cross inoculation experiments in the greenhouse and farmers' fields, involving inoculation treatments on shrub and crop legumes indicated that some of the indigenous isolates excel (over +N control, elite national and imported inoculum strains) in their symbiotic performances. Also, "need for inoculation" studies indicated that legume crops markedly differ in their nodulation at various locations, demonstrating differences in the occurrence of compatible rhizobia at various locations and benefits from inoculation. These results, while suggesting the enormous untapped rhizobial resources resident in Ethiopian soils, indicate ample opportunities for selecting elite strains to enhance effective Rhizobium-legume symbiosis in agroecosystems. This work has formed the basis for expansion of N2Africa (www.n2africa.org) and intensification of efforts to enhance symbiotic nitrogen fixation in the farming systems of Ethiopia.

11:30-11:45 Presenter: Matthew Denton (University of Adelaide, Australia)

A national survey of farmers in Australia to understand rhizobial inoculant use for pulse and pasture legumes

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Inoculation of legumes with rhizobia is crucial to improving nitrogen fixation by pulse and pasture legumes but there is very little information on the inoculation practices of Australian farmers. A survey was conducted in 2013 to explore grower knowledge and practice in relation to rhizobial inoculation. The survey was completed by 405 growers, and represented a farmed area of just over 1 million hectares, across major cropping regions in Australia. Results showed that approximately 90% of mungbean and chickpea areas sown were inoculated. In contrast, less than 50% of lentil crops were inoculated. For pasture legumes, 85% of lucerne area was inoculated, while less than 50% of vetch, subterranean clover and annual medic pastures were typically inoculated. Peat formulation was by far the most common method of application (82% of respondents). Other formulations were also important, including granules (19%) and freeze-dried formulations (14%). A substantial proportion of farmers used more than one type of formulation. Farmers generally had a reasonable knowledge about rhizobia and their use, although ten per cent did not understand rhizobial specificity for particular legumes. Areas of concern are the acceptance of mixing inoculant with fertilisers (22% of farmers) or pesticides (9%). Ninety percent of

survey respondents reported that they had used inoculants on legumes in recent years. The key reasons provided for using inoculants related to perceived soil nitrogen benefits, improvements in yield and the influence of agronomic advisers. Of the ten per cent that did not inoculate, over half specified that inconvenience was a reason and also that the benefits of inoculation were not clear.

11:45-12:00 Presenter: Adriana Navarro-Borrell (Agriculture and Agri-Food Canada, Swift Current)

Managing soil microbial resources and wheat yield through crop rotation in 4-year systems in the Canadian Prairie

Navarro-Borrell, A.^{1,2*}, Hamel, C.^{1,2}, Gan, Y.², and Germida, J.¹. ¹Department of Soil Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8; ²Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, SK, S9H 3X2. *(adriana.navarroborrell@agr.gc.ca)

Crop rotation is a key component of enhancing agroecosystems performance, but it is unknown how different legume-cereal rotation systems may impact soil fungal communities and crop productivity. In this study, (1) we determined the relative efficiency of eight crop rotation systems involving pea, lentil, chickpea and wheat, based on crop performance, and (2) defined the contribution of plant-associated fungi to system performance. Pea-wheat-lentil-wheat, lentil-wheat-lentil-wheat, and pea-wheat-pea-wheat best promoted wheat seed yield and increased seed carbon content. In the lentil-wheat-lentil-wheat, and pea-wheat yield. Wheat monoculture and chickpea-wheat-wheat-wheat were the least productive systems. In year 3, diversified rotations had higher levels of mycorrhizal root colonization than rotations involving growing wheat on wheat. However, in year 4, wheat roots were similarly colonized by AM and non-AM fungi in all rotation systems.

The influence on plant growth of the soil microbiota selected under the first three phases of the rotation systems tested in the field was evaluated in an inoculation study, in the greenhouse. The microbiota selected under the rotation lentil-wheat-chickpea best promoted plant biomass and yield. Inoculation with soil from the plots under chickpea-wheat-chickpea and lentil-wheat-chickpea also increased wheat biomass, but the microbiota selected under wheat monoculture did not promote wheat growth; rather, it led to the lowest wheat biomass production. Yield was higher than the control in all the treatments, except pea-wheat-wheat-wheat and wheat monoculture.

We conclude that more diversified rotations promote AM fungal associations in the field and can enhance wheat productivity.

12:15-12:30 Presenter: Navid Bazghaleh (University of Saskatchewan)

Genotypic variation in the response of chickpea to arbuscular mycorrhiza and fungal endophytes

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Chickpea roots form symbioses with arbuscular mycorrhizal (AM) fungi and non-AM fungal endophytes

that influence its growth and productivity. Evidence shows that the genotype of the host plant can impact the outcome of root symbioses on plant growth, suggesting that intraspecific variations in the functionality of root symbioses may exist in chickpea. Here, we tested 13 cultivars of chickpea on the function of root symbioses formed by the AM, and non-AM endophytic fungi inoculated individually or co-inoculated. The AM symbiosis increased the biomass and nitrogen fixation activity of most cultivars of chickpea, whereas non-AM fungal endophytes had negative to positive influences. The genotype of both the chickpea and the fungal colonizer influenced the function of the symbioses. The root symbioses promoted plant growth most effectively in CDC Cory, CDC Anna and CDC Frontier and stimulated nitrogen fixation in CDC Corrine. Coinoculation had additive effects on CDC Corrine, CDC Anna and CDC Cory but non-AM fungal endophytes reduced the positive effect of AM fungi on Amit and CDC Vanguard. Our results indicate that there is genetic variation in the response of chickpea to AM and non-AM fungal symbiosis. This variation could allow the selection of genotypes that form efficient symbioses with the AM and non-AM fungal endophytes, using conventional breeding techniques.

Thursday, July 10, 2014

Biotic Stress and Plant Microbe Interactions

ICLGG Symbiosis

Chair: Kirstin Bett (University of Saskatchewan, Canada)

11:00-11:30 Presenter: Pascal Ratet (Institut des Sciences du végétal CNRS, Gif sur Yvette, France)

Identification of *Medicago truncatula* genes preventing development of plant defenses during symbiosis

Berrabah, F.¹, Cayrel, A.¹, Eschstruth, A.¹, Bourcy, M.¹, Wen, .², Mysore, K.², Gourion, B.¹, and Ratet, P.^{1*}. ¹Institut des Sciences du végétal CNRS, 91198 Gif sur Yvette, France; ²Samuel Roberts Noble Foundation, Ardmore, OK, USA. *(Pascal.Ratet@isv.cnrs-gif.fr)

The symbiosis between *Rhizobium* bacteria and legume plants results in the formation of nitrogen-fixing nodules on the roots of the host plant. In these nodules host large amount of bacteria that fix nitrogen for the benefit of the plant. How the plant can accommodate the chronic infection without eliciting defense reactions and how rhizobia can be distinguished from pathogenic bacteria during the post infection stages is not well understood. We have characterized the *dnf2* and *symCRK M. truncatula* mutants blocked in the symbiotic process after bacterial infection of the nodule symbiotic cells (Bourcy et al., 2013, New Phytol 197, 1250-61; Berrabah et al., 2014, submited). Nodules formed by the mutants contain only few layers of infected cells. Furthermore, they exhibit defense-like reactions which clearly contrast with premature senescence frequently observed during inefficient symbioses. The defense reactions are supported by the induction of molecular markers, the accumulation of phenolic compounds in the nodules and the death of the bacterial partner. In contrast to *symCRK*, the *dnf2* mutant phenotype is conditional suggesting a control of the symbiotic organ immunity by the environment. The description of these two mutants constitutes a significant step toward the elucidation of the mechanisms responsible for the establishment of the chronic infection during symbiosis.

11:30-11:50 Presenter: Georgina Hernandez (Universidad Nacional Autónoma de México, Mexico)

MicroRNAs from common bean (*Phaseolus vulgaris*) in the rhizobia symbiosis and in the response to abiotic stress

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Common bean (*Phaseolus vulgaris*) is the most important grain legume for human consumption; it establishes N-fixing symbiosis (SNF) with rhizobia. Plant microRNAs (miRNAs) (21-22 nt non-coding RNAs) are key post-transcriptional regulators of different processes. Our goal is to contribute deciphering common bean miRNA functions.

High-throughput sequence led to identify 114 known miRNAs in *P. vulgaris* (Peláez et al.2012). We are mapping these miRNAs in the *Phaseolus* genome (www.phytozome.net, v.1.0) and predicting novel MiRNA genes.

Aluminum toxicity (Al-t) is widespread in acidic soils where the common bean is produced and it is a limiting factor for crop production and for SNF. We have identified some 30 miRNAs that respond to Al-t in roots and nodules of SNF bean plants and have performed target gene expression analysis (Mendoza-Soto et al. submitted).

We have selected common bean miRNAs for functional analysis. The regulation of copper homeostasis and biotic interactions by miR398b has been demonstrated. The two validated miR398b target genes: CSD and novel Nod19 are up-regulated, while miR398b is down-regulated, under oxidative stress resulting from Cu toxicity, early interaction with rhizobia or infection of pathogenic fungi (Naya et al. 2014). Also we demonstrated the crucial role of miR172c and AP2-1 target gene in bean root and nodule development and in nodule function during effective N-fixing symbiosis. The over-expression of bean miR172c results in increased root growth, earlier nodulation, increased nodule number and higher nitrogenase activity (Nova-Franco et al. submitted).

11:50-12:10 Presenter: Jeremy Murray (John Innes Centre, Norwich, UK)

The Rhizobial Infectome: Uncovering the Genes that Control the Early Steps of the Legume-Rhizobia Interaction.

Breakspear, A.¹, Liu, C.¹, Wang, M.², He, J.², Cerri, M.R.³, Guan, D.¹, Roy, S.¹, Stacey, N.¹, Rogers, C.¹, Trick, M.¹, Morieri, G.¹, Oldroyd, G.E.D.¹, Niebel, A.³, Downie, J.A.¹, de Carvalho-Niebel, F.³, and Murray, J.D.^{1*}. ¹John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom; ²Cancer Genomics Research Laboratory, NCI Leidos Biomedical Research, Inc. 8717 Grovemont Circle Gaithersburg, MD 20877, USA; ³LIPM, INRA-CNRS, Unité Mixte de Recherche 441/2594, 31326 Castanet-Tolosan, France. *(jeremy.murray@jic.ac.uk)

Sinorhizobium meliloti infects its host Medicago truncatula through root hairs which is essential for the

eventual development of nitrogen-fixing nodules. This process depends on flavonoids released from the plant roots that induce the production of signalling molecules called Nod factors by the rhizobia. These Nod factors are host-symbiont specific and essential throughout the infection process. To gain insight into the early-stage interactions we isolated and transcriptionally profiled root hairs from WT seedlings at different time points during infection. We also profiled several mutants: *sickle* which is hyperinfected, and *nin, ern1* and *nfya1*, that have defects in infection thread formation. The data indicate that that plant likely produces and secretes a large spectrum of compounds from root hairs including specific Nod factor inducing molecules, but also Nod factor degrading enzyme, strigolactones, phytoalexins, and potentially glucose. The data also provided insights into specific processes that are involved in infection thread growth and development, in particular the regulation of hormone biosynthesis and signalling. A gene regulatory network based on a larger data set that included the mutants was constructed which provided specific insights into how some of these components are controlled.

12:10-12:30 Presenter: Hongyan Zhu (University of Kentucky, USA)

Genetic control of symbiosis specificity in the legume-rhizobial mutualism

Zhu, H.^{1*}. ¹University of Kentucky. *(hzhu4@uky.edu)

Legume plants are able to engage in root nodule symbiosis with nitrogen-fixing soil bacteria, collectively called rhizobia. This mutualistic association is highly specific, such that each rhizobial species/strain interacts with only a specific group of legumes, and vice versa. Symbiosis specificity can occur at multiple phases of the interaction, ranging from initial bacterial attachment and infection to late nodule development associated with nitrogen fixation. Genetic control of symbiosis specificity is complex, involving fine-tuned signal communication between the symbiotic partners. We will present our recent work on positional cloning of the soybean genes that control nodulation specificity with different rhizobial strains. We will also report our progress in cloning genes in Medicago that regulate symbiosis specificity at the nitrogen-fixing phase.

IFLRC Disease and Pest Resistance

Chair: Carlota Vaz Patto (Universidade Nova de Lisboa, Portugal)

11:00-11:30 Presenter: Weidong Chen (USDA/ARS, Washington State University, USA)

What roles do fungal secondary metabolites play in interactions between *Ascochyta* fungi and cool season food legumes?

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Fungal plant pathogens produce many secondary metabolites including many that are toxic to plants (phytotoxins). Some of these phytotoxins are host-selective (toxic only to particular genotypes of host

plants) and required for pathogenicity, while many others are non host-selective and toxic to many different plants. The Ascochyta blight pathogens of cool season food legumes produce an array of non hostselective toxins. Although Ascochyta blight pathogens are generally host-specific, their phytotoxins are not. Nevertheless, these phytotoxins have often been proposed to be virulence/pathogenicity factors without conclusive evidence. We are currently investigating the roles of secondary metabolites produced by the Ascochyta blight pathogens in their pathogenicity and ecology. We found that each Ascochyta species produces a unique set of secondary metabolites, which is highly correlated to evolutionary relationships inferred among the species. For the chickpea blight pathogen, all isolates of Ascochyta rabiei produce solanapyrone toxins, which are unique among Ascochyta species, but identical to those produced by the potato pathogen, Alternaria solani. During studying the roles of solanopyrones in pathogenesis, the solanapyrone synthase gene in both Ascochyta rabiei and Alternaria solani was disrupted through targeted gene replacement. The resulting mutants do not produce solanapyrones, but accumulate the precursor presolanopyrone which is not toxic to plants. Surprisingly, these solanapyrone-deficient mutants are equally pathogenic, if not more pathogenic, than the wild-type strains, on their respective hosts. Results show that the solanapyrone toxins are not required for pathogenicity, but may play important roles in the biology of these fungi including competition and survival in nature.

11:30-11:45 Presenter: Aurore Desgroux (INRA, Rennes, France)

Identification of pea lines resistant to Aphanomyces euteiches and related root architecture traits

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Common root rot caused by *Aphanomyces euteiches*, is a major soil borne disease of pea in many countries. Genetic resistance is considered to be the main way to control the disease. The role of plant root architecture in Aphanomyces root rot resistance is not well known. This study aimed at identifying pea lines with high levels of resistance in a collection of 175 accessions enriched with sources of resistance and root architectural traits harbored by resistant lines. The collection was assessed for resistance to *A. euteiches* on the roots and on the aerial plant parts, both in controlled conditions and in a four-year and multi-location field disease network. The collection was also described for root architectural traits on healthy and infested plants in controlled conditions at young plant stage. Lines with higher levels of resistance than partially resistant controls were identified. Susceptible and partially resistant plants mostly showed a decrease of lateral root density and dry weight, suggesting their ability to preserve the root system in response to infection. These results will be confirmed in the field using a set of contrasted genotypes. Genome wide association analysis will be performed to compare the genetic control of Aphanomyces root rot resistance and of root architecture traits in response to infection.