# Multiscale modelling of the emergence of virulent virus populations: towards new breeding strategies for building durable virus resistance

Frederic Fabre, Claude Bruchou, Alain Palloix, Hervé Lecoq, Benoît Moury


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# 10th International Plant Virus Epidemiology Symposium

## *Controlling Epidemics of Emerging and Established Plant Virus Diseases - The Way Forward*

15-19 October 2007, ICRISAT
Patancheru 502324, AP, India

Program and Abstracts

*Organized and Hosted by the* International Crops Research Institute for the Semi-Arid Tropics

# 10th International Plant Virus Epidemiology Symposium

## *Controlling Epidemics of Emerging and Established Plant Virus Diseases - The Way Forward*

15 - 19 October 2007
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Hyderabad, Andhra Pradesh, India

## Program and Abstracts

*Compiled by*
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**About ICRISAT**

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political international for science based agricultural development. ICRISAT conducts research on sorghum, pearl millet, chickpea, groundnut and pigeonpea – crops that support the livelihoods of the poorest of the poor in the semi-arid tropics encompassing 48 countries. ICRISAT also shares information and knowledge through capacity building, publications, and information and communication technologies. Established in 1972, ICRISAT belongs to the Alliance of Centers supported by the Consultative Group on International Agricultural Research (CGIAR) [www.icrisat.org; www.cgiar.org].

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International Plant Virus Epidemiology (IPVE) Group is a coordinated by the IPVE Committee of the International Society of Plant Pathology (ISPP). The ISPP was founded in 1968 in the United Kingdom to sponsor the development of plant pathology worldwide. The ISPP sponsors the International Congress of Plant Pathology, and International Meetings of its Subject Committees. To date the IPVE Committee has conducted nine international symposia in different parts of the world. This $10^{th}$ IPVE Symposium is the first to be held in Asia.

The Previous IPVE Symposia were held in:

1. Oxford, UK, 28 - 31 July 1981
2. Corowa, Australia, 25 - 27 August 1983
3. Orlando, USA, 6 - 8 August 1986
4. Montpellier, France, 1 - 5 September 1989
5. Valenzano (Bari), Italy, 27-31 July 1992
6. Jerusalem, Israel, 23 - 28 April 1995
7. Almeria, Spain, 11 - 16 April 1999
8. Ascherleben, Germany, 12 - 17 May 2002
9. Lima, Peru, 4 - 7 April 2005

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# *Participants of the 10$^{th}$ IPVE Symposium*

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# Overview of the Program

**14 October 2007: Sunday: Academic Court**

Arrivals, registration, conference kit distribution

**15 October 2007: Monday: RWC Auditorium**

| | |
|---|---|
| 08.15-09.00 | Registration and conference kit distribution |
| 09.00-10.15 | Session - I: Inauguration |
| 10.15-11.00 | Group photograph & Refreshment break |
| 11.00-12.40 | Session - II: Epidemiology & Evolution |
| 12.40-13.40 | Lunch break (Banquet hall) |
| 13.40-15.20 | Session - III: Emerging viruses |
| 15.20-15.50 | Refreshment break & Poster Session - I (Academic court) |
| 15.50-17.30 | Session - IV: Viruses of Cereal Crops & Soil-Borne Viruses |
| 17.30-18.30 | Poster Session - I (Academic court) |
| 18.30-19.00 | Cultural show |
| 19.00 onwards | Dinner at Patio |

**16 October 2007: Tuesday: RWC Auditorium**

| | |
|---|---|
| 08.30-09.50 | Session - V: Plant Biosecurity & Modelling |
| 09.50-10.20 | Refreshment break & Poster Session - II (Academic court) |
| 10.20-11.20 | Session - V: Plant Biosecurity & Modelling |
| 11.20-13.30 | ICRISAT overview and lunch at sunset park |
| 13.30-15.10 | Session - VI: Virus-Vector Evolution & Interactions |
| 15.10-15.40 | Refreshment break & Poster Session - II (Academic court) |
| 15.40-17.10 | Session - VI: Virus-Vector Evolution & Interactions |
| 17.10-18.00 | Session - VII: IPVE Committee General Session |
| 18.00-19.00 | Poster Session - II (Academic court) |
| 19.00 onwards | Dinner at Marry Cummings Park |

**17 October 2007: Wednesday: Concurrent Sessions**

| | **Great Lakes Conference Hall** | **New Sahel Conference hall** |
|---|---|---|
| 08.30-10.00 | Session - VIII: Advances in Virus Disease Management | Session - IX: Characterization and Diagnosis of Viruses & Vectors |
| 09.50-10.30 | Refreshment break & Poster Session – III (Academic court) | |
| 10.30-13.20 | Continuation of Session - VIII | Continuation of Session - IX |
| 13.20-14.20 | Lunch at Banquet hall | |
| 14.30 onwards | City tour | |

**18 October 2007: Thursday: RWC Auditorium**

| | |
|---|---|
| 08.30-10.00 | Session - X: Molecular Epidemiology and Ecology |
| 10.00-10.50 | Refreshment break & Poster session – IV (Academic court) |
| 10.50-13.10 | Session - X: Molecular Epidemiology and Ecology |
| 13.10-14.15 | Valedictory Lunch at Banquet hall |

**19 – 20 October 2007: Field Tour to Aurangabad**

# Detailed Program

**15 October 2007: Monday (Ralf W. Cummings Auditorium)**

*08.15-09.00* — *Registration and conference kit distribution*

*09.00-10.15* — ***Session I: Inaugural Session***
***Presiding Chair: Prof. N. Rishi,***
President, Indian Virological Society

*Introduction* — ***P. Lava Kumar,*** Convener, IPVE Symposium, IITA

*Welcome message* — ***Farid Waliyar,*** *Chair, Organizing Committee, ICRISAT*

*Inaugural Address* — ***W.D Dar*** *(Director General) &* ***DH Keatinge*** *(Deputy Director General), ICRISAT*

*IPVE Chairman's Address* — **Roger A.C. Jones**, Chair, IPVE Committee
Agricultural Research Western Australia
Topic: Plant viruses at the ancient ecosystem - recent agroecosystem interface

*Remarks by Presiding Chair* — ***N. Rishi***

*Vote of thanks* — ***I. Nagaraj,*** *HR Director, ICRISAT*

10.15-11.00 — **Group photograph & Refreshment break (Academic court)**

*11.00- 12.40* — **Session II: Epidemiology and Evolution**
**Co-Chairs. H. Lecoq and C.L.L. Gowda**

*11.00-11.40*
*Keynote*
**Microevolutionary dynamics of *Rice yellow mottle virus*: studies at the interface of epidemiology and evolution**
D. Fargette[1], A. Pinel-Galzi[1], O. Traoré[2], F. Sorho[3], S. Rakotomalala[4], E. Sangu[5], Z. Kanyeka[5], Y. Séré[3], E. Hébrard[1] and G. Konaté[2]; *[1]IRD, 34394 Montpellier, France; [2]INERA, Laboratoire de Virologie et Biotechnologie Végétale, Ouagadougou, Burkina Faso; [3]Africa Rice Center (WARDA) Cotonou, Bénin; [4]FOFIFA, Mahajanga, Madagascar; [5]University of Dar es Salaam, Dar es Salaam, Tanzania*

*11.40-12.10*
*Keynote*
**Plant virus epidemics**
J.M. Thresh; *Natural Resources Institute, University of Greenwich, Chatham, Kent, UK*

*12.10-12.40*
*Keynote*
**Thrips-transmitted *Iris yellow spot tospovirus* epidemics in the US: progress and challenges in unraveling the epidemiological factors underlying the disease outbreaks in onion seed and bulb crops**
H.R. Pappu[1], R. Sampangi[2], S.K. Mohan[2], H.F. Schwartz[3] and S.I. Rondon[4]; *[1]Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430, USA; [2]University of Idaho, Parma Research & Extension Center, Parma, ID 83660, USA; [3]Department of Bioagricultural Sciences and Pest Management, Colorado State University, Ft. Collins, CO 80523, USA; [4]Oregon State University, Hermiston Agricultural Research and Extension Center, Hermiston, OR 97838, USA*

12.40-13.40 — **Lunch at Banquet Hall**

*13.40-15.20* — **Session III: Emerging viruses**
**Co-Chairs: R.A.C. Jones and R. van der Vlugt**

*13.40-14.10*
*Keynote*
**Evidence of novel viruses by analysis of nucleic acids in virus-like particle fractions from *Ambrosia psilostachya***
Ulrich Melcher[1], Vijay Muthukumar[1], Michael J. Palmer[1], Graham Wiley[2] and Bruce Roe[2]; *[1]Oklahoma State University, Stillwater; [2]University of Oklahoma, Norman, Oklahoma, USA*

*14.10-14.30*
**Pospiviroid infections in ornamental plants and their potential risks for vegetable crops**
J.Th.J. Verhoeven, C.C.C. Jansen, A.W. Werkman and J.W. Roenhorst; *Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, the Netherlands*

*14.30-14.50* **Tomato torrado virus and Tomato marchitez virus, new plant picorna-like viruses infecting tomato**
M. Verbeek[1], A.M. Dullemans[1], P.C. Maris[2], J.F.J.M. van den Heuvel[2] and R.A.A. van der Vlugt[1]; [1]*Plant Research International BV, PO Box 16, 6700 AA Wageningen, The Netherlands;* [2]*De Ruiter Seeds BV, P.O. Box 1050, 2660 BB Bergschenhoek, The Netherlands*

*1450-15.20* **Current status of tospoviruses infecting vegetable crops in India**
K.S. Ravi[1], Suresh Kunkalikar[1], M. Bhanupriya[1], P. Sudarsana[1], Prem Rajagopalan[1], Usha B. Zehr[1] and R.A. Naidu[2]; [1]*Molecular Virology, Mahyco Research Center, Dawalwadi, Post Box no 76, Jalna-Aurangabad Road, Maharashtra – 431 203, India;* [2]*Department of Plant Pathology, Washington State University, Prosser, WA 99350, USA*

1520-1550 **Refreshment break & poster viewing (Academic court)**
*15.50-17.30* **Session IV: Viruses of Cereal Crops and Soil-borne Viruses**
**Co-Chairs: T. Kuhne and Hanu R. Pappu**

*15.50-16.20* *Keynote* **Thirty years of research on soil-borne viruses in cereals – the past and the present in Europe**
Thomas Kühne; *Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, D-06484 Quedlinburg, Germany*

*16.20-16.50* *Keynote* **Importance of seed and soil-borne transmission in the spread of pecluviruses**
C. Bragard[1], B. Dieryck[1], G. Otto[1], A. Legrève[1] and P. Delfosse[2*]; [1]*Unité de Phytopathologie, U.C.L., Croix du Sud, 2 bte 3 1348 Belgium;* [2]*Virology, ICRISAT Sahelian center, Sadoré, Niger; * Present address: Centre de Recherche Public - Gabriel Lippmann, L-4422 Belvaux, Luxemburg*

*16.50-17.10* **Cereal viruses in the Czech Republic: distribution, detection, genetic diversities and resistance**
J. Kumar-Kundu[1], J. Vacke[1], G. Červená[2], J. Chrpová[3] and V. Šíp[3]; [1]*Department of Virology, Crop Research institute, Prague, 161 06 Czech Republic;* [2]*Diagnostic lab of State Phytosanitary Administration, Šlechtitelů 23/773, 779 00; Olomouc Czech Republic;* [3]*Department of Plant Breeding Methods, Crop Research institute, Prague, 161 06 Czech Republic*

*17.10-17.30* **Evaluation of the appearance of different genotypes of *Wheat dwarf virus* in Germany**
J. Schubert[1], A. Habekuß[2], K. Katzmeyer[3] and Holger Jeske[3]; *Federal Centre for Breeding Research on Cultivated Plants,* [1]*Institute of Resistance Research and Pathogen Diagnostics and* [2]*Institute of Epidemiology and Resistance Resources, Erwin-Baur-Str. 27, 06484 Quedlinburg;* [3]*Institute of Biology, Department of Molecular Biology and Plant Virology, University of Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart; Germany*

***17.30-18.30*** ***Poster Session I (Academic court)***
***18.30-19.00*** ***Cultural show (Ralf. W. Cummings Auditorium)***
***19.00*** ***Dinner at Patio***

Poster Session - I (15 October 2007)

PP-1_01 **Rhizomania in Iran, a disease under strong selection pressure**
M. Mehrvar[1,2] and B.C. Bragard[2]; [1]*Department of Plant Protection, School of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran;* [2]*Unite de phytopathologie,.U.C.L., B-1348 Louvain-la-Neuve, Belgium*

PP-1_02 **Development of post-transcriptional gene silencing (PTGS) constructs against *Groundnut bud necrosis virus***

Pranav Chettri, P.U. Krishnaraj and M.S. Kuruvinashetti; *Institute of Agri-Biotechnology, University of Agricultural Sciences, Krishinagar, Dharwad -580 005, Karnataka, India*

PP-1_03 **Host range of *Beet curly top virus* (BCTV) in Khorasan and Hamadan provinces in Iran**
Nasser Beikzadeh[1], Aziz Bagheri[2] and Judith K. Brown[3]; [1]*Shahid Hasheminejad Higher Education Center, Mashad, Iran;* [2]*Khorasan Agricultural and Natural resources Research Center, Hamadan, Iran;* [3]*Department of Plant Sciences, University of Arizona, USA*

PP-1_04 **Experiments on tuber necrotic ringspot isolates of *Potato virus Y* (PVY$^{NTN}$) in tomato in Hungary**
A.P. Takács[1], K. Salánki[2], R. Szücs[1], L. Palkovics[3], G. Kazincz and J. Horváth[1]; [1]*Pannon University of, Georgikon Faculty for Agriculture, H-8361 Keszthely, P.O. Box 71, Hungary;* [2]*Agricultural Biotechnology Center, H-2101 Gödöllő, P.O. Box 170, Hungary;* [3]*Corvinus University of Budapest, Department of Plant Pathology; H-1118 Budapest, Hungary*

PP-1_05 **Pre-exposure of calli to ozone promotes systemic resistance of the regenerated *Capsicum annuum* cv. PKM1 (Chili) plantlets against *Cucumber mosaic virus* attack**
N. Sudhakar, D. Nagendra-Prasad, S. Periyar selvam, P.M. Balu, S. Krishna Kumar, C. Raghunandanan, N. Mohan and K. Murugesan; *Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, India*

PP-1_06 ***Banana bunchy top virus*: an increasing threat to banana production in Pakistan**
Javaria Qazi, Imran Amin, Shahid Mansoor and Rob Briddon; *National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O Box 577, Faisalabad, Pakistan*

PP-1_07 **Weeds as a source for inoculum of viruses and potential threat to cultivated crops**
Muhammad Mubin, Khadim Hussain, Mazhar Hussain and Shahid Mansoor; *Plant Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Jhang Road, Faisalabad, Pakistan*

PP-1_08 **First report of *Tobacco streak virus* on *Cosmos bipinnatus* Cav. and *Impatiens balsamina* L. from India**
N. Arun Kumar, Arti S. Kitkaru, Usha B. Zehr and K.S. Ravi; *Mahyco Research Center, Dawalwadi, PB- 76, Jalna-Aurangabad Road, Maharashtra – 431 203 , India*

PP-1_09 **Tomato torrado virus – new virus transmitted by greenhouse whitefly (*Trialeurodes vaporariorum*) in Poland**
H. Pospieszny, N. Borodynko, B. Hasiow, A. Obrepalska-Steplowska, M. Budziszewska; *Institute of Plant Protection, Department of Virology and Bacteriology, Miczurina 20, Poznan, Poland*

PP-1_10 **Incidence and diversity analysis of *Prunus necrotic ring spot virus* infecting *Prunus* sp. in the North-Western Himalayas, India**
V. Chandel, T. Rana, V. Hallan and A.A. Zaidi; *Plant Virology Lab, Institute of Himalayan Bioresource Technology, Palmapur-176 061, Himachal Pradesh, India*

PP-1_11 **Studies on symptomatology and host range of a virus causing necrotic wilt in sunflower**
C.V. Deepa Rani, D.D. Nirmal, Prashant L. Sontakke and S.D. Somwanshi; *Dept. of Plant Pathology, College of Agriculture, Marathwada Agri. University, Parbhani-431402, India*

PP-1_12 **Comparison of suppressiveness of *Cucumber mosaic virus* infection by foliar spray of compost and vermicompost mixtures in *Lycopersicon esculentum* cv. PKM1**
B. Ravindran[1], N.Sudhakar[2], G. Sekaran[1] and K. Murugesan[2]; [1]*Central leather research institute, adyar, Chennai-20, India;* [2]*Centre for advanced studies in Botany, Guindy campus, university of Madras, Chennai-25, India*

PP-1_13 **Incidence of plant virus in Antarctica**
I.G. Budzanivska, S.V. Dolgorukova and V.P. Polischuk; *Taras Shevchenko' Kyiv National University, 01033 Kyiv, Ukraine*

PP-1_14 **Spread of *Plum pox virus* in Ukraine**
L. Yusko[1], H. Snihur[2], I. Budzanivska[2], O. Afonina[2], and V. Polischuk[2]; *[1]Transcarpathian territorial centre of the plant quarantine, Institute of plants protection UAAS, 21 Universytetska St., Uzhgorod 88017, Ukraine; [2]Virology Department, Taras Shevchenko' Kyiv National University, 64 Volodymyrska st., Kyiv 01033, Ukraine*

PP-1_15 **Natural occurrence of *Dasheen mosaic virus* in aroid tuber crops and foliage ornamentals in Andhra Pradesh, India**
M. Padmavathi[1], K. Navodayam[2], J.K. Prasadji[2] and P.Sreenivasulu[1]; *[1]Department of Virology, Sri Venkateswara University, Tirupati – 517 502, A.P, India; [2]Agricultural Research Station, Acharya NG Ranga Agricultural University, Kovvur – 534 350, West Godavari, A.P, India.*

PP-1_16 **Biological and molecular characterization of *Pea seed-born mosaic virus* of important faba bean fields of Iran**
B. Rohani, M. Koohi Habibi, Gh. Mosahebi, N. Hamzeh, K. Ghazanfari and S. Hosseini; *Department Of Plant Protection, Faculty Of Agriculture, University Of Tehran, Karaj, Iran*

PP-1_17 **Mixed viral infection in sunflower plants**
G.M. Orlovska and A.L. Boyko; *Virology department, Taras Shevchenko National University of Kyiv, 64 Volodymyrska st., Kyiv 01033, Ukraine*

PP-1_18 **The new highly pathogenic strain of *Potato virus X***
M.V. Arkhipenko, N.A. Nikitin, A.A. Smirnov, V.K. Novikov, N.P. Rodionova, O. V. Karpova and J.G. Atabekov; *Department of virology, Moscow State University, Moscow, Russia*

PP-1_19 **Prevalence of potato viruses in the Punjab state, India**
S.S. Kang, Ashok Kumar and S.K. Thiara; *Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141004, Punjab, India*

PP-1_20 **Variability of *Sri Lankan cassava mosaic virus* (SLCMV) in Tamil Nadu, India**
N. Rajinimala[1], R. Rabindran[1] and S. Mohan[1]; *Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore – 641003, Tamil Nadu, India*

PP-1_21 **Occurrence and characterization of *Bean common mosaic virus* on vanilla (*Vanilla planifolia* Andrews) in India**
V. Bhadra Murthy and A.I. Bhat; *Crop Protection Division, Indian Institute of Spices Research, Marikunnu (PO), Calicut 673012, Kerala, India*

PP-1_22 **Emerging diseases caused by begomoviruses in sub-temperate regions in India**
Y. Kumar, V. Hallan and A.A. Zaidi; *Plant Virology Lab, Institute of Himalayan Bioresource Technology, Palmapur-176 061, Himachal Pradesh, India*

PP-1_23 **Spread of virus diseases of fruit crops in Ukraine**
V.M. Udovychenko, N.V. Tryapitcyna, S.O. Vasyuta, T.V. Medvedeva, V.A. Maliyenko and K.M. Udovychenko; *Institute of Horticulture, UAAS, Sadova str.,6, Kyiv 03027, Ukraine*

PP-1_24 **The role of olfaction in differential settling by the green peach aphid on *Potato leaf roll virus*-infected and non-infected potato plants**
Esther Ngumbi, Sanford D. Eigenbrode, Hongjian Ding and Nilsa A. Bosque-Pérez; *University of Idaho, Department of Plant Soil and Entomological Sciences, P.O. Box 442339, Moscow, ID 83844-2339, USA*

PP-1_25 **The contribution of using healthy looking planting material in the control of sweet potato virus disease under the subsistence sweet potato production in Uganda**
S.B. Mukasa, E. Gasura and R.J. Rukarwa; *Department of Crop science, Makerere University, P.O. Box 7062, Kampala, Uganda*

PP-1_26 **Major viral diseases incidence on important vegetable crops in Hyderabad, India**
S.K. Sain and M.L. Chadha; *AVRDC-Regional Center for South Asia, ICRISAT, Patancheru, 502324, India*

PP-1_27 **Association of a *Begomovirus* complex with yellow mosaic disease of jute in India**
Raju Ghosh, Sujay Paul, Subha Das, Paramita Palit, Sanchalika Acharyya, Javid Iqbal Mir, Anirban Roy, and Subrata Kumar Ghosh; *Plant Virus Laboratory and Biotechnology Unit, Division of Crop Protection, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata – 700120, West Bengal, India*

PP-1_28 **Biological differentiation of furoviruses in cereals**
Ute Kastirr, Fred Ehrig and T. Kühne; *Federal Centre for Breeding Research on Cultivated Plants, Institute of Resistance Research and Pathogen Diagnostics, Erwin-Baur-Strasse 27, D-06484 Quedlinburg Germany*

PP-1_29 **Occurrence of furoviruses in winter barley**
Ute Kastirr, Fred Ehrig and Thomas Kühne; *Federal Centre for Breeding Research on Cultivated Plants, Institute of Resistance, Research and Pathogen Diagnostics, Erwin-Baur-Straße 27, D-06484 Quedlinburg, Germany*

PP-1_30 ***Plum pox virus* epidemiology in nursery blocks of *Prunus* under high inoculum pressure**
E. Vidal, A. Moreno, E. Bertolini, N. Capote, M. Gil, C. Collado and M. Cambra; *Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera km 5, 46113 Moncada, Spain*

PP-1_31 **Influence of an alternate weed host, *Solanum sarrachoides* (sendtner) on the epidemiology of *Potato leaffroll virus* in potato ecosystems of Idaho**
Rajagopalbabu Srinivasan[1] and Juan M. Alvarez[1], SD. Eigenbrode[2] and Nilsa A. Bosque-Pérez[2]; *[1]1693S 2700W, Aberdeen Research and Extension Center, University of Idaho, Idaho-USA; [2]Department of Plant Soil and Entological Sciences, University of Idaho, Moscow, Idaho, USA*

PP-1_32 **Occurrence of some viruses and viroids on stone fruits in the Czech Republic**
P. Rysanek, M. Zouhar, L. Grimova, M. Hassan and H. Ketta; *Department of Plant Protection, Czech University of Life Sciences, 165 21 Prague, Czech Republic*

PP-1_33 **Sweet potato leaf curl disease – a new emerging virus problem of sweet potato in India**
T. Makeshkumar, B.S. Prakash Krishnan, V. Hegde, M.L. Jeeva and S. Edison; *Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, India*

PP-1_34 **Occurrence and distribution of viruses infecting fluted pumpkin (*Telfairia occidentalis*, Hook F.) in Imo State, Nigeria**
T.T. Oben[1,2], G.I. Atiri[1], O. Fagbola[3], S.A. Akinbade[2], P.O. Oyibo[4] and P. Lava Kumar[2*]; *[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria; [2]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; [3]Department of Agronomy, University of Ibadan, Ibadan, Nigeria; [4]Federal University of Technology, Owerri, Nigeria*

**PP-1_35** **Incidence and distribution of virus and virus-like diseases on yams (*Dioscorea* sp.) in the Republic of Benin**
A.O. Eni[1,2], J.d'A. Hughes[1†], R. Asiedu[1], R. Bandyopadhyay[1], P. Lava Kumar[1] and M.E.C. Rey[2]; *[1]International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan PMB5320, Nigeria;*

[2]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa; [†]Current address: Asian Vegetable Research and Development Centre (AVRDC), Shanhua, Taiwan

### 16 October 2007: Tuesday: (Ralf W. Cummings Auditorium)

*08.30-11.50* **Session V: Biosecurity and Modelling**
**Co-Chairs: Ravi. K. Khetarpal and F. Nutter, Jr.**

*08.30-09.10 Keynote* **The role of plant biosecurity in preventing and controlling emerging plant virus disease epidemics**
B. Rodoni; *Department of Primary Industries Victoria, Knoxfield centre, 621 Burwood Highway Knoxfield, 3156, Victoria Australia*

*09.10-09.50 Keynote* **Use of GPS, GIS, and geostatistics to develop pre-plant virus disease prediction models**
F.W. Nutter, Jr., E. Byamukama and A. Robertson; *Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames IA, 50011, USA*

09.50-10.20 **Refreshment break & poster viewing (Academic court)**

*10.20-10.40* **Role of epidemiology of seed-transmitted viral diseases for developing seed certification standards for grain legumes in India**
V. Celia Chalam[1], R.K. Khetarpal[1], H.S. Prakash[2] and Ashok Mishra[3]; *[1]Division of Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi-110 012, India; [2]Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Mysore-570 006, Karnataka, India; [3]Jain Irrigation Systems Ltd., Agripark, Jain Hills, Shirsoli Road, Jalgaon, Maharashtra, India*

*10.40-11.00* **A survey of grapevine viruses: epidemiology from an instant picture**
F. Javier Legorburu[1], Elena Recio[1], Enrique López[2], José Baigorri[2], Miguel Larreina[2], Laura Caminero[3], Félix Cibriain[3] and Faustino Aguirrezábal[3]; *[1]NEIKER- Basque Institute for Agricultural Research and Development, Apartado 46, E-01080 VITORIA/GASTEIZ, Basque Country, Spain; [2]Servicio de Viticultura y Enología, Diputación Foral de Álava, Cra. de Lapuebla de Labarca s/n, E-01300 LAGUARDIA, Basque Country, Spain; [3]EVENA-Estación de Viticultura y Enología de Navarra, Valle de Orba 34, E-31390 OLITE, Navarre, Spain*

*11.00-11.20* **Landscape patterns of aphid vectored viruses of pea in the Palouse region of Idaho and Washington: implications for virus forecasting**
Sanford D. Eigenbrode, Melia Nafus and Alexander Karasev; *University of Idaho, Plant Soil and Entomological Sciences, Ag Sci 242, Moscow, Idaho 83844-2339, USA*

*11.20–13.30* **ICRISAT overview & Lunch at sunset park**

*13.30-17.10* **Session VI: Virus-Vector Evolution & Interactions**
**Co-chairs: S. Blanc and A. Fereres**

*13.30-14.10 Keynote* **Helper-dependency in vector-transmission: ecological role of a complex process too often adopted by plant viruses**
Stéphane Blanc; *UMR BGPI, INRA-CIRAD-AgroM, Bat TA 41/K, Campus International de Baillarguet, 34398 Montpellier cedex 05, France*

*14.10-14.50 Keynote* **Behavioural aspects of virus transmission by Hemipteran insects**
Alberto Fereres, Marcelo Pedreira de Miranda[1] and Miguel Cid; *Departamento de Protección Vegetal. Instituto de Ciencias Agrarias- ICA. Consejo Superior de Investigaciones Cientificas. CSIC. C/Serrano 115 dpdo. 28006 Madrid, Spain; [1]Departamento de Entomologia, Fitopatologia e Zoologia Agrícola -ESALQ/USP, Piracicaba, Brasil*

*14.50-15.10* **Testing of the vector dependency hypothesis with aphids and whiteflies**
S.J. Castle; *Arid-Land Agricultural Research Center, USDA-ARS, 21881 N. Cardon Lane, Maricopa, AZ 85238, USA*

15.10-15.40 ***Refreshment break & Poster Session – II (Academic court)***

*15.40-16.10 Keynote* **Mixed-viral infections (Potato virus Y-Potato leafroll virus) affect the biology and preference of aphid vectors and consequently the epidemiology of potato viruses**
J.M. Alvarez and R. Srinivasan; *Department of Plant Soil and Entomological Sciences (PSES), University of Idaho, Aberdeen R & E Center, 1693 S. 2700 W, Aberdeen, ID 83210, USA*

*16.10-16.30* **Evidences for transmission of *Indian cassava mosaic virus* through *Bemisia tabaci* - cassava biotype**
Binu Antony, M.S. Palaniswami, T. Makeshkumar and Annie John[1]; *Central Tuber Crops Research Institute, Thiruvananthapuram 695 017 Kerala, India; [1]BMT Wing, Thiruvananthapuram Kerala, India*

*16.30-16.50* ***Risk of spread and vector relations of* Potato yellow vein virus *in the Andes***
I. Barker[1], H. Gamarra[1], S. Fuentes[1], G. Müller[1], H. Juarez[1] and F. Morales[2]; *[1]International Potato Center (CIP), Apartado 1558, Lima 12, Peru; [2]International Center for Tropical Agriculture (CIAT). Apartado Aéreo 6713, Cali, Colombia*

*16.50-17.10* **Reducing the global impact of thrips-transmitted tospoviruses in diverse cropping systems: successes gained and challenges that lie ahead**
H.R. Pappu[1], B. Mandal[2], R.K. Jain[2], A. S. Csinos[3], A. K. Culbreath[3] and D. G. Riley[4]; *[1]Department of Plant Pathology, Washington State University, Pullman, USA; [2]Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India; [3]Department of Plant Pathology, and [4]Entomology, University Georgia, Tifton, USA*

***17.10-18.00*** **Session VII: IPVE Committee General Session**
**Chair: R.A.C. Jones**

*17.10-17.25* *-Introduction*

*17.25-17.40* **The Plant Virus Ecology Research Coordination Network**
Ulrich Melcher[1], Yan Song[1], Tony Bridgewater[1] and Carolyn Malmström[2]; *[1]Oklahoma State University, Stillwater, Oklahoma; [2]Michigan State University, East Lansing, Michigan, USA*

*17.40-18.00* *-IPVE business issues*
***18.00–19.00*** ***Poster Session - II (Academic court)***
***19.00*** ***Dinner at Marry Cummings Park***

Poster Session - II (16 October 2007)

PP-2_36 **Quantifying yield losses caused by plant viruses**
F.W. Nutter, Jr. and E. Byamukama; *Department of Plant Pathology, Iowa State University, Ames IA, USA*

PP-2_37 **Possible ways of transmission of sugar beet viruses**
N. Senchugova and O.Postoenko; *Virology Department, National Taras Shevchenko University of Kyiv, Ukraine.*

PP-2_38 **Distribution of sunflower viruses in four provinces of Iran**
S. Hosseini, G. Mosahebi and M. Koohi Habibi; *Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran*

PP-2_39 **Occurrence of *Lettuce mosaic virus, Cucumber mosaic virus* and *Tomato spotted wilt virus* on lettuce**
P. Soleimani; College of Agriculture, Islamic Azad University, Dezful Branch, Iran

PP-2_40 **Studies on monitoring and assessment of crop loss to necrosis virus disease in sunflower**
Y.D Narayana[1] and D.S. Chandra Mohan[2]; *[1]University of Agricultural Sciences, Dharwad, Karnataka, India; [2]Syngenta India Pvt Ltd., Aurangabad, Maharashtra, India*

PP-2_41 **Shoot bug, *Peregrinus maidis* (Ashmead), a vector of sorghum stripe disease: trend in Karnataka during post rainy season on sorghum**
A.R. Hundekar, R.A. Balikai, B.D. Biradar and G.M. Sajjanar; *University of Agricultural Sciences, Dharwad, Regional Agricultural Research Station Bijapur- 586 101, Karnataka, India*

PP-2_42 **Loss estimation due to shoot bug, *Peregrinus maidis* (Ashmead) in *rabi* sorghum under field conditions**
R.A. Balikai, Raju Anaji, B.D. Biradar, G.M. Sajjanar and A.R. Hundekar; *University of Agricultural Sciences, Dharwad, Regional Agricultural Research Station, Bijapur- 586 101, Karnataka, India*

PP-2_43 **Serological relationship of vegetable infecting tospoviruses in India**
P. Sudarsana[1], Arti S. Kitkaru[1], Suresh Kunkalikar[1], Usha B. Zehr[1], Shyi-Dong Yeh[2], S. Adkins[3], K.S. Ravi[1] and R.A. Naidu[4]; *[1]Mahyco Research Center, Dawalwadi, Post Box no 76, Jalna-Aurangabad Road, Maharashtra -, India; [2]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan R.O.C; [3]U.S. Department of Agriculture- Agriculture Research Service, 2001, Fort Piece, FL,; [4]Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Washington State University, WA 99350, USA*

PP-2_44 **Evaluation of pigeonpea genotypes for resistance to sterility mosaic disease**
Shivam[1], V.B. Chauhan[1], P. Lava Kumar[2]* and R.B. Singh[1]; *[1]Banaras Hindu university, Varanasi, India; [2]International crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, AP, India;*Current address: International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan PMB5320, Nigeria*

PP-2_45 ***Pepino mosaic virus*: epidemiology, economic impact and pest risk analysis (PEPEIRA)**
R.A.A. van der Vlugt; *Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands*

PP-2_46 ***Apple chlorotic leaf spot virus*: Incidence, epidemiology, genomic diversity and strategies for disease management**
T. Rana, V. Chandel, V. Hallan and A.A. Zaidi; *Plant Virology Lab, Institute of Himalayan Bioresource Technology, Palmapur-176 061, Himachal Pradesh, India*

PP-2_47 **Dissemination of viruses of cereal crops in agrocoenosises of Ukraine**
H. Snihur[1], V. Polischuk[1] and U. Kastirr[2]; *[1]Department of Virology, Taras Shevshenko' Kyiv National University, Kyiv, Ukraine; [2]Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, D-06484 Quedlinburg, Germany*

PP-2_48 **Quantifying the temporal and spatial dynamics of plant viruses: a quadrat-based approach**
E. Byamukama[1], A. Robertson[1], D. Nordman[2] and F.W. Nutter, Jr[1]; *[1]Department of Plant Pathology, Department of Plant Pathology, 351 Bessey Hall; [2]Department of Statistics and statistical laboratory, Snedecor Hall, Iowa State University, Ames IA, 50011, USA*

PP-2_49 **Transmission and physical properties of virus causing sunflower necrosis**
C.V. Deepa Rani[1], D.D. Nirmal[2], Sunita Magar[3] and Mirza F.N. Baig[4] ; *Department of Plant Pathology, College of Agriculture, Marathwada Agricultural University, Parbhani-431 402, Maharashtra, India*

PP-2_50 **Development of diagnostic assays for the detection and identification of parental and recombinant PVY RNAs**
V.W. Fomitcheva[1] and J. Schubert[1]; [1]*Institute of Resistance Research and Pathogen Diagnostics, Federal Centre for Breeding Research on Cultivated Plants, Erwin-Baur-Str 27, 06484 Quedlinburg, Germany*

PP-2_51 **Tobacco transgenic rootstock harboring silencing construct against ZYMV coat protein protect wt tobacco from viral infection**
D. Wolf[1], A. Zelcer[1], D. Liebman[2], R. Gour[1] and A. Gal-on[2]; [1]*Institute of Plant Science,* [2]*Institute of Plant Protection, The Volcani Center, Agriculture Research Organization, P.O. Box 6, Bet-Dagan, 50250, Israel*

PP-2_52 **Tests for molecular diagnosis of *Citrus yellow mosaic virus* infecting sweet orange: a step in controlling the movement of infected budwood**
A.M. Anthony Johnson[1], Basanta borah[2], Indranil Dasgupta[2] and D.V.R. Sai Gopal[1]; [1]*Department of Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India;* [2]*Department of Plant Molecular biology, University of Delhi South campus, New Delhi, India*

PP-2_53 **Identification of soybean viral mosaic disease with IC-RT-PCR method in Lorestan province**
Arezoo Naghavi[1,2] , M. Koohi habibi[3], R. Farokhi-nejad[1] and M. Hashemi[4]; [1]*Department of Plant Protection, Faculty of Agriculture Shahid Chamran University, Ahvaz, Iran;* [2]*Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khoramabad, Iran;* [3]*Department of Plant Protection, Tehran University, Karaj, Iran;* [4]*Seed and plant improvement institute*

PP-2_54 **Delay of *Cucumber mosaic virus* infection in strobilurin treated tomato plants**
C. Varveri[1], M. Christopoulou[1], E. Markellou[1], N. Vassilakos[1], A. Tzima[1] and D. Servis[2]; [1]*Benaki Phytopathological Institute, 8 S. Delta st., 145 61 Kifissia, Greece;* [2]*BASF Agro Hellas S.A, 48 Aigialeias st., 151 25 Paradeisos Amarousiou, Greece*

PP-2_55 **Identification and differentiation of viruses in asparagus plantings in Germany**
F. Rabenstein[1], J. Schubert[1], and A. Habekuß[2]; *Federal Centre for Breeding Research on Cultivated Plants,* [1]*Institute of Resistance Research and Pathogen Diagnostics and* [2]*Institute of Epidemiology and Resistance Resources, Erwin-Baur-Str 21, 06484 Quedlinburg, Germany*

PP-2_56 **Quantitation by direct squash real-time RT-PCR of RNA targets acquired by single aphids**
A. Moreno, E. Bertolini, N. Capote, A. Olmos, A. Hermoso de Mendoza and M. Cambra; *Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera km 5, 46113 Moncada, Spain*

PP-2_57 **Role of stage of plant infection by *Bean common mosaic virus* on yield and further seed transmission of the virus in black gram and green gram**
Dinesh Chand, V. Celia Chalam and R.K. Khetarpal; *Division of Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi-110 012, India*

PP-4_58 **First sequence based evidence for occurrence of a recombinant *Begomovirus* and a satellite β-DNA associated with leaf curl disease of kenaf (*Hibiscus cannabinus*) in northern India**
Sujay Paul[1], Raju Ghosh[1], Subha Das[1], Paramita Palit[1], Sanchalika Acharyya[1], Javid Iqbal Mir[1], Sujata chaudhury[2], Anirban Roy[1], and Subrata Kumar Ghosh[1]; [1]*Plant Virus Laboratory and Biotechnology Unit, Division of Crop Protection, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata – 700 120, West Bengal, India;* [2]*Department of Botany, University of Kalyani, Kalyani, Nadia 741 235, India*

PP-2_59 **Different virus and virus like diseases infecting *Lilium*, Tulip and Alstroemeria in Himachal Pradesh, India**
Ashutosh Bhaik, Anil Handa, Usha Sharma, P.D. Thakur, Kumud Jarayal and Promil Kapoor; *Plant virology laboratory, Department of Mycology and Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni Solan-173 230, Himachal Pradesh, India*

PP-2_60 **Epidemiological studies of different meteorological factors and vector population in the development of virus disease(s) in bell pepper and its management using cultural practices**
Promil Kapoor, P.D. Thakur and Ashutosh Bhaik; *Plant Virology Laboratory, Department of Mycology and Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni Solan, Himachal Pradesh, India*

PP-2_61 **Selective targeting of RNAi pathyway by viral suppressors**
Priyanka Singh[1], Satendra K. Mangrauthia[2], Vikas Koundal[1], Shelly Praveen[1]; [1]*Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110 012, India;* [2]*Division of Biochemistry, Indian Agricultural Research Institute, New Delhi-110 012, India*

PP-2_62 **Sequence diversity in the coat protein (CP) gene of *Papaya ringspot virus* and development of CP gene constructs for papaya transformation**
Surekha Agarwal[1], Sudeep Bag[1], Y.K. Jaiswal[2] and R.K. Jain[1*]; [1]*Unit of Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi – 110 012, India;* [2]*Department of Biotechnology, School of studies, Jiwaji University, Gwalior – 474011, India*

PP-2_63 **Survey and serodiagnosis of viruses infecting papaya (*Carica papaya* L.) plants in Uttar Pradesh, India**
Shyam Singh and L.P. Awasthi; *Department of Plant Pathology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad-224 229 (U.P.), India*

PP-2_64 **Biological, serological and molecular characterization of chrysanthemum isolate of *Cucumber mosaic virus***
S. Kumar and S.K. Raj*; *Plant Molecular Virology Group, Centre for Plant Molecular Biology, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, (U.P.) India*

PP-2_65 **Epidemiology of virus causing necrotic wilt causing sunflower (*Helianthus annuus L.*)**
D.D. Nirmal[1], Prashant L. Sontakke[2], K.T. Apet[3] and Sunita J. Magar[4]; *Department of Plant Pathology, College of Agriculture, Marathwada Agricultural University, Parbhani-431 402, Maharashtra, India*

PP-2_66 **Incidence of *Tobacco streak virus* on Bt. cotton under natural condition**
G.P. Jagtap[1], Prashant L. Sontakke[2] and P.V. Khalikar[3]; *Department of Plant Pathology, College of Agriculture, Marathwada Agricultural University, Parbhani-431 402, India*

PP-2_67 **Integrated management of *Papaya ringspot virus* (PRSV) in Peru**
J. Tenorio[1], J. Salazar[2], S. Fuentes[1], C. Aguilar[1], C. Malpartida[1], G. Müller[1], C. Chuquillanqui[1], A. Cabrera[1], C. De La Torre[1], J. Marín[2], E.V. Campoverde[1], I. Barker[1] and L. F. Salazar[1,3]; [1]*International Potato Center (CIP). Apartado 1558, Lima 12, Peru;* [2]*Servicio de Sanidad Vegetal (SENASA), Lima, Peru;* [3]*Agdia Inc., IN, USA*

PP-2_68 ***Chickpea chlorotic stunt virus* affecting cool-season food legumes in West Asia and North Africa**
Safaa G. Kumari[1], Khaled Makkouk[1], Nader Asaad[1], Nouran Attar[1] and Mai Hlaing Loh[2]; [1]*Virology Laboratory, International Center for Agriculture Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria;* [2]*Department of Primary Industries (DPI), 621 Burwood Highway, Knoxfield, Victoria, Australia, 3180*

PP-2_69 **Breeding for resistant varieties to control rice virus disease in Korea**
D.Y. Kwak, J.H. Lee, U.S. Yeo, D.S. Park, C.S. Kim, M.S. Shin, B.G. Oh and J.K. Kim; *Yeongnam Agricultural Research Institute, NICS, RDA, Milyang 627-803, Korea*

PP-2_70 **Frequency of mixed infection of *Tobacco streak virus* and *Peanut bud necrosis virus* in groundnut**
A.S. Reddy, P. Lava Kumar[†], K. Subrahmanyam, F. Waliyar and S.N. Nigam; *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; [†]Current address: International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria*

PP-2_71 **Incidence of seed transmitted viruses in cowpea and soybean in Nigeria**
J.U. Mgbechi-Ezeri, S.A. Akinbade, J.d'A. Hughes[†], R. Bandyopadhyay and P. Lava Kumar; *International Institute of Tropical Agriculture (IITA), P.M.B. 5320, Ibadan, Nigeria; [†]Current address: Asian Vegetable Research and Development Centre (AVRDC), Shanhua, Taiwan*

## 17 October 2007: Wednesday (Concurrent sessions)

| Time | Session VIII: Advances in Virus Disease Management<br>*RWC Auditorium*<br>Co-Chairs: A. Varma and K. Makkouk | Session IX: Characterization and Diagnosis of Viruses & Vectors<br>*S.M. Barghouti Conference Hall*<br>Co-chairs: I. Barker and M. Fletcher |
|---|---|---|
| *08.30-13.10* | | |
| *08.30 –09.10*<br>*Keynote* | *08.30-09.10*<br>*Natural resistance mechanisms to viruses in plants*<br>G. Loebenstein, Y. Elad and Diana Leibman; *Agricultural Research Organization, Bet Dagan, Israel* | *08.30 – 08.50*<br>**The identity of the leafhopper vectors in the genus *Orosius* Distant (Hemiptera: Cicadellidae)**<br>Murray J. Fletcher[1] and Andrew Mitchell[2]; *[1]NSW Dept Primary Industries, Orange, NSW Australia; [2]NSW Dept Primary Industries, Wagga Wagga, NSW Australia*<br><br>*08.50 – 09.10*<br>**Control of vectors and insecticide resistance: Implications for Disease control**<br>Nilima Prabhaker[1], Steven J. Castle[2] and Nick C. Toscano[1]; *[1]Department of Entomology, University of California, Riverside, CA, USA; [2]U.S. Arid-Land Agricultural Research Center, USDA-ARS, Maricopa, AZ, USA* |
| *09.10-09.30* | **A new pathotype of *Pepper mild mottle virus* (PMMV) overcomes the $L^4$ resistance genotype of pepper cultivars**<br>Y. Antignus, O. Lachman, M. Pearlsman, L. Maslenin and A. Rosner; Department of Virology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel | Diagnosis, epidemiology and management of *Watermelon bud necrosis virus* infecting watermelon<br>M. Krishnareddy, S. Jalali and R. Venugopalan; *Division of Plant Pathology, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore-560 089, India* |
| *09.30-09.50* | **Multiscale modelling of the emergence of virulent virus populations: towards new breeding strategies for building durable virus resistance** | **Transmission and epidemiology of *Papaya ringspot virus-w* (PRSV-W) infecting pumpkin**<br>N.K. Krishna Kumar, C.M. |

F. Fabre[1], C. Bruchou[2], A. Palloix[3], H. Lecoq[1] and B. Moury[1]; [1]*INRA, Unité Pathologie Végétale, F-84000 Avignon, France;* [2]*INRA, Unité Biostatistique & Processus spatiaux, F-84000 Avignon, France;* [3]*INRA, Unité Génétique & Amélioration des Fruits & Légumes, F-84000 Avignon, France*

Kalleshwaraswamy, S. Saroja, M. Krishna Reddy and R. Venugopalan; *Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore-560 089, Karnataka, India*

09.50-10.30 **Refreshment break & Poster Session- III (Academic court)**

**Session VIII: Advances in Virus Disease Management,**
***RWC Auditorium***
**Co-chairs: J.M. Thresh and S.N. Nigam**

*10.30-11.00 Keynote*

**Epidemiology and integrated management of persistently aphid-transmitted legume and cereal viruses in West Asia and North Africa**
Khaled M. Makkouk and Safaa G. Kumari; *Virology Laboratory, International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria*

*11.00-11.20*

**Development and evaluation of transgenic groundnut for resistance to *Tobacco streak virus* (TSV)**
K. Saivishnupriya[1], S. Arockiasamy[1], P. Lava Kumar[1†], A.S. Reddy[1], Arun K. Nalla[1], F. Waliyar[1], S.N. Nigam[1], Roger N. Beachy[2] and K.K. Sharma[1]; *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India;* [2]*Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis MO 63132, USA. †Current address: International Institute of Tropical Agriculture, Ibadan, Nigeria*

*11.20-11.40*

**Transcomplementation and synergism in plants: implications for viral transgenes?**
Jonathan R. Latham and Allison K. Wilson; *Bioscience Resource Project, PO Box 66, Ledbury, Herefordshire HR8 9AE, UK*

*11.40-12.00*

**Management of the island sugarcane planthopper, *Eumetopina flavipes***

**Session IX: Characterization and Diagnosis of Viruses & Vectors**
***S.M. Barghouti Conference Room***
**Co-chairs: B. Rodoni and G. Loebenstein**

*10.30-11.00*

**Epidemiology of leafhopper-borne *Maize yellow stripe virus* in Egypt**
Aboul-Ata E. ABOUL-ATA[1], A.M. Abdel-Kader[1], M.M. El-Bolok[2] and E.D. Ammar[2]; [1]*Department of Plant Virus and Phytoplasma Research, Plant Pathology Research Institute, Agricultural Research Center, P.O. Box 12619, Giza, Egypt;* [2]*Department of Entomology and Pesticides, Faculty of Agriculture, Cairo University, Egypt*

*11.00-11.20*

**Emergence of new phytoplasma and virus diseases**
Karl Maramorosch; *Department of Entomology, 93 Lipman Drive, School of Environmental and Biological; Sciences, Rutgers- The State University of New Jersey, New Brunswick, NJ 08901, USA*

*11.20-11.40*

**Comparison of high throughput PCR and tissue blot immunoassay for large-scale virus field surveys**
Angela J. Freeman[1], Merrin E. Spackman[1], Mohammad Aftab[1], Brendan C. Rodoni[2], Mai-Hlaing Loh[2] and Joop Van Leur[3] ; [1]*Department of Primary Industries, Private Bag 260, Horsham, VIC 3401, Australia;* [2]*Department of Primary Industries, Private Bag 15, FGDC, VIC 3156, Australia;* [3]*NSW Department of Primary Industries, Tamworth, NSW 2340, Australia*

*11.40-12.00*

**Development of a diagnostic multiplex IC-RT-PCR system for the differentiation**

**(Delphacidae), vector of Ramu Stunt disease of sugarcane in Papua New Guinea**
Kylie L. Anderson[1], Mohamed Sallam[2], Robert C. Magarey[3] and Bradley C. Congdon[1]; *[1]School of Marine and Tropical Biology, James Cook University, McGregor Road, Smithfield, Queensland, Australia 4878; [2]BSES Limited, PO Box 122, Gordonvale, Queensland, Australia, 4865; [3]BSES Limited, PO Box 566, Tully, Queensland, Australia, 4854*

**of *Potato virus Y* strains**
V.W. Fomitcheva[1], J. Schubert[1], K. Lindner[2], J. Fletcher[3] and J. Sztangret-Wiśniewska[4]; *[1]Institute of Resistance Research and Pathogen Diagnostics, Federal Centre for Breeding Research on Cultivated Plants, Erwin-Baur-Str 21, 06484 Quedlinburg, Germany; [2]Institute of Plant Virology, Microbiology and Biosafety, Federal Biological Research Centre for Agriculture and Forestry, Messeweg 11/12, 38104 Braunschweig, Germany; [3]New Zealand Institute for Crop & Food Research Ltd., Private Bag 4704, Christchurch, New Zealand; [4]Plant Breeding and Acclimatization Institute, Radzików, Department of Młochów, Młochów, Poland*

*12.00-12.20*

**The challenges and potential options for controlling virus diseases of legumes and tuber crops in West Africa**
P. Lava Kumar, J.U. Mgbechi-Ezeri, S.A. Akinbade, A.O. Eni, R. Asiedu, A.G.O. Dixon, C. Fatokun and R. Bandyopadhyay; *International Institute of Tropical Agriculture (IITA), Ibadan, PMB 5320, Nigeria*

**Occurrence and PCR detection of cassava mosaic geminiviruses on *Jatropha curcus* in Tamil Nadu**
R. Rabindran, D. Ladhalakshmi, N. Rajinimala, N. Ragupathi, S. Bharathi, R. Vimalkanth, V. Prakasam and R. Samiyappan; *Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India*

*12.20-12.40*

**Centennial of research on groundnut rosette disease: what is known and what still needs to be known to achieve effective control of this menace in Sub-Saharan Africa**
F. Waliyar[1], P. Lava Kumar[1†], M. Osiru[2], E. Monyo[2], B.R. Ntare[3] and S.N. Nigam[1]; *[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; [2]ICRISAT, P.O. Box 1096, Lilongwe, Malawi; [3]ICRISAT, BP320, Bamako, Mali; [†]Current address: International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria*

**Genetic diversity of *Banana bunchy top virus* in India**
R. Selvarajan, V. Balasubramanian, T. Rajesh, N. Lakshmi Dhevi, R. Rajmohan and M.M. Mustaffa; *National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India*

*12.40-13.00*

**Screening groundnut varieties for resistance to the groundnut rosette disease**
E.S. Monyo, M.O. Osiru, F. Waliyar[1] and H.J. Charlie; *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), P.O. Box 1096, Lilongwe, Malawi. [1]ICRISAT, Patancheru 502 324, Andhra Pradesh, India*

**Molecular characterization of viral double-stranded RNA and plasmid-like DNA from the plant pathogenic fungus *Rhizoctonia solani***
N. Bharathan, Kristy Anthony and Seema Bharathan; *Department of Biology, Indiana University of Pennsylvania, Indiana, Pennsylvania, USA*

*13.00-13.20*

**Good agricultural practices (GAPs) for successful management of *Papaya***

**Legume yellow mosaic viruses, genetically isolated begomoviruses:**

***ringspot virus* (P) in papaya – a case study of IARI regional station, Pune, India**
S.K. Sharma, V.M. Chavan, U.M. Kadam, S.P.S. Tomar and M.G. Dhale; *Indian Agricultural Research Institute, Regional Station, Agricultural College Estate, Shivajinagar, Pune -411 005, India*

**diversity of legume-infecting begomoviruses in Pakistan**
Muhammad Ilyas, Javaria Qazi, Shahid Mansoor and RobW. Briddon; *Plant Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan*

*13.20-14.20* **Lunch break**
14.20 ***City tour (Dinner in city on participants)***

Poster Session – III (17 October 2007)

PP-3_72 **Evaluation of FHIA hybrids for resistance to *Banana bunchy top virus* and yield potential in Malawi**
B.M.L. Mwenebanda[1], L.H. Mwamlima[2] and T. Msosa[2] and D.L.N. Banda[3]; [1]*IITA/SARRNET, Chitedze Research Station, P.O. Box 30258, Lilongwe 3, Malawi;* [2] *Mkondezi Research Station, P.O. Box 133, Nkhata-Bay, Malawi;* [3]*Bvumbwe Research Station, P.O. Box 5748, Limbe, Malawi*

PP-3_73 **Response of selected candidate resistant summer squash cultivars to *Zucchini yellow mosaic virus* (ZYMV) infection**
Jiri Svoboda and Jaroslav Polak; *Crop Research Institute, Drnovska 507, 161 06 Prague 6, Czech Republic*

PP-3_74 **Effect of *Cotton leaf curl virus* (ClCuV) on reproductive ability of *Bemisia tabaci***
R.S. Mann[1], J.S. Sidhu[2], N.S. Butter[2], A.S. Sohi[2] and P.S. Sekhon[2]; [1]*University of Florida, Gainesville, Fl, USA- 32611-0620;* [2]*Punjab Agricultural University, Ludhiana. Punjab, India*

PP-3_75 **Negative effects of cotton leaf curl virus on host suitability and longevity of *Bemisia tabaci***
J.S. Sidhu[1], R.S. Mann[2], N.S. Butter[1], A.S. Sohi[1] and P.S. Sekhon[1]; [1]*Punjab Agricultural University, Ludhiana, Punjab, India 141004;* [2]*University of Florida, Gainesville, Florida, USA*

PP-3_76 **Bud necrosis of watermelon (WBNV) and associated species complex of thrips**
H.R. Ranganath, Vikas Kumar and N.K. Krishna Kumar; *Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore 560 089, India*

PP-3_77 **A novel tool to study the epidemiology of *Cucumber vein yellowing virus* (CVYV) and *Cucurbit yellow stunting disorder virus* (CYSDV) in cucurbit crops using a Real Time RT-PCR assay**
F.M. Gil-Salas[1], J. Morris[2], D. Janssen[1], G. Budge[2], A. Colyer[2], N. Boonham[2] and I.M. Cuadrado[1]; [1]*Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (I.F.A.P.A., C.I.C.E.), Junta de Andalucía, 04745 La Mojonera, Almeria, Spain;* [2]*Central Science Laboratory, Sand Hutton, York Y041 1LZ, UK*

PP-3_78 **Identification and ecology of thrips species, the vectors of tospoviruses in India**
Vikas Kumar, H.R. Ranganath, N.K. Krishna Kumar and R. Asokan; *Division of Entomology & Nematology, Indian Institute of Horticultural Research Hessaraghatta, Bangalore, India*

PP-3_79 **Sensitive broad spectrum detection and quantification tools for managing peanut clump disease**
B. Dieryck[1], A. Legrève[1], P. Delfosse[2] and C. Bragard[1]; [1]*Unité de Phytopathologie, U.C.L., Croix du Sud, 2 bte 3 1348 Belgium;* [2]*Virology, ICRISAT Sahelian center, Sadoré, Niger*

PP-3_80 **A generic (RT)-PCR test for caulimoviruses**
A.M. Dullemans and R.A.A. van der Vlugt; *Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands*

PP-3_81 **Induction of systemic resistance in *Lycopersicon esculentum cv.*PKM1 against *Cucumber mosaic virus* by using plant growth promoting rhizobacteria (PGPR)**
N. Sudhakar, S.L. Dinesh., S. Sudha., S. Kumaran and K. Murugesan; *Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, India*

PP-3_82 **Production of polyclonal antiserum against the RTBV CP gene expressed in *E. coli* for the detection of *Rice tungro bacilliform virus***
D. Ladhalakshmi[1], R. Rabindran[1], I. Dasgupta[2], M. Bharathi[3], R. Velazhahan[1] and T.S. Raveendran[4]; [1]*Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore – 3, Tamil Nadu, India;* [2]*Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India;* [3]*Department of Agricultural Entomology, TNAU, Coimbatore-3, TN, India;* [4]*Centre for Plant Breeding and Genetics, TNAU, Coimbatore-3, TN, India*

PP-3_83 **Serodiagnosis of mixed infection of *Ilar* and *Tospo* viruses in Mungbean in Tamil Nadu, India**
J. Vinod[1], T. Ganapathy[1], R. Rabindran[1] and M. Bharathi[2]; [1]*Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University (TNAU), Coimbatore-3, Tamil Nadu, India;* [2]*Department of Agricultural Entomology, Centre for Plant Protection Studies, TNAU, Coimbatore-3, Tamil Nadu, India*

PP-3_84 **Influence of temperature on the transmission of luteoviruses by apterous aphids**
A. Habekuß, E. Schliephake and F. Ordon; *Federal Centre for Breeding Research on Cultivated Plants, Institute of Epidemiology and Resistance Resources, Erwin-Baur-Straße 27, D-06484 Quedlinburg, Germany*

PP-3_85 **Screening diploid banana germplasm against *Banana streak virus* by PCR and NASH**
R. Selvarajan, V. Balasubramanian, S. Uma and M.M. Mustaffa; *National Research Centre for Banana, Tiruchirapalli, India*

PP-3_86 **Effect of plant extracts and derivatives, butter milk and virus inhibitory chemicals on watermelon mosaic virus infection in cucumber**
Anjana Shukla, Sarika Srivastava and J.P. Tiwari; *Plant Pathology Lab, M.L.K.P.G. College, Balrampur (U.P.), India*

PP-3_87 **Management of tomato leaf curl virus disease and its whitefly vector on tomato (*Lycopersicon esculentum* Mill) in eastern Uttar Pradesh, India**
Sarika Srivastava, Anjana Shukla, J.P. Tiwari and G.P. Srivastava; *Plant Pathology Lab, M.L.K. (P.G) College, Balrampur, Uttar Pradesh, India*

PP-3_88 **Detection and management for viral disease complex of soybean**
J. Duria, T. Ganapathy and R. Rabindran; *Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, India*

PP-3_89 **Identification of *Cotton leaf curl Rajasthan virus* infecting tomato in Pakistan**
Muhammad Shafiq Shahid, Shahid Mansoor and Rob W. Briddon; *National Institute for Biotechnology and Genetic Engineering, P.O Box 577 Jhang Road, Faisalabad, Pakistan*

PP-3_90 ***Ageratum enation virus* causes yellow vein disease of *Sonchus oleraceus***
Muhammad Tahir[1], Faiza Saleem[1], Deeba Noreen Baig[1], Muhammad Saleem Haider[1], Naeem Rashid[1], Javed Iqbal[1], Muhammad Akhtar[1] and Rob W. Briddon[2]; [1]*School of biological Sciences, University of the Punjab, New Campus Lahore;* [2]*National Institute for Biotechnology and Genetic Engineering, Jhang Road, Faisalabad, Pakistan*

PP-3_91 **Occurrence of cucurbit viruses in Punjab, India**
Abhishek Sharma[1], SK Thiara[2], SS Kang[2] and Sumit Inder Kaur[2]; [1]*Department of Vegetable Crops, Punjab Agricultural University, Ludhiana-141 004, India;* [2]*Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141 004, India*

PP-3_92 **Development of coat protein gene mediated resistance to *Tobacco streak virus* in groundnut**
Sudeep Bag[1], R.S. Singh[2] and R.K. Jain[1]; [1]*Unit of Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, 110 012, India;* [2]*Department of Biotechnology, Punjabi University, Patiala, 147 001, India*

PP-3_93 **Molecular detection, DNA-1 based sequence identities and phylogenetic analysis of five Indian isolates of *Banana bunchy top virus***
Radha Vishnoi and S.K. Raj; *Molecular Virology Lab, National Botanical Research Institute, Lucknow-, India*

PP-3_94 **Detection of phytoplasma in ornamental and economically important plants**
S.K. Snehi, M.S. Khan and S.K. Raj; *Molecular Plant Virology, National Botanical Research Institute, Lucknow- 226001 (U. P.) India*

PP-3_95 **Correlation of environmental factors in relation to PVX and PVY disease severity and aphid population**
Kamra Mahmood, M. Usman Ghazanfar and Shahbaz Talib Sahi; *Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*

PP-3_96 **Recent advances in breeding for resistance to viruses in legumes: lessons for pigeonpea**
D.A. Odeny; *Max Planck Institute for Plant Breeding Research, Carl-von-Linné Weg 10, Cologne, Germany*

PP-3_97 **Persistence and infectivity of an Iranian isolate of *Tomato yellow leaf curl virus* in whitefly (*Bemesia tabaci*)**
J. Mozafari[1], A. Azizi[1,2] and M. Shamsbakhsh[2]; [1]*National Plant Gene bank, Seed & Plant Improvement Institute, Mahdasht Road, Karaj, Iran; 2. Department of Plant Pathology, Tarbiat Modares University, Tehran, Iran*

PP-3_98 **Production of monoclonal antibodies against *Cowpea mottle virus***
S.A. Akinbade, T.T. Oben, J.U. Mgbechi-Ezeri and P. Lava Kumar; *International Institute of Tropical Agriculture (IITA), P.M.B. 5320, Ibadan, Nigeria*

**18 October 2007: Thursday (Ralf W. Cummings Auditorium)**

*08.30-13.10* **Session X: Molecular Epidemiology and Ecology**
**Co-chairs: D. Fargette and Y. Antignus**

*08.30-09.00 Keynote* **The molecular epidemiology of cucurbit viruses**
H. Lecoq and C. Desbiez ; *INRA, station de Pathologie Végétale, Domaine Saint Maurice, BP94, 84140 Montfavet, France*

*09.00-09.20* **Molecular epidemiology of *Watermelon mosaic virus* in France and evolution of viral populations**
Cécile Desbiez, Benoit Joannon, Catherine Rys and Hervé Lecoq; *INRA, Station de Pathologie Végétale, Domaine Saint Maurice, Montfavet cedex, France*

*09.20-09.40* **The molecular basis of *Zucchini yellow mosaic virus* symptom expression**
Y. M. Shiboleth[1], E. Haronsky[1], D. Leibman[1], T. Arazi [2], D. Wolf[2], A. Zelcer[2], V Gaba[1] and A.

Gal-On[1]; *Institutes of Plant Protection[1] and Plant Sciences[2]; The Volcani Center, Agricultural Research Organization, Bet-Dagan 50250, Israel*

*09.40-10.00* **Strains of *Potato virus Y* in Dutch seed potato culture**

R.A.A. van der Vlugt[1], M. Verbeek[1], P.G.M. Piron[1], C. Cuperus[1], G. van den Bovenkamp[2] and E. de Haan[2]; [1]*Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands;* [2] *Nederlandse Algemene Keuringsdienst (NAK), P.O. Box 1115, 8300 BC Emmeloord, The Netherlands*

10.00-10.50 **Refreshment Break & Poster Session – IV (Academic court)**

**Co-chairs: U. Melcher and S. Winter**

*10.50-11.20 Keynote* **Emergence and reemergence plant viruses in India: impact and management options**

Anupam Varma; *Advanced Center for Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi - 110 012, India*

*11.20-11.50 Keynote* **Variability is the arsenal that sustains war between plants and pathogens**

K. Muralidharan; *Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, India*

*11.50-12.10* **A diversity study of Cassava brown streak virus(es) infecting cassava in East Africa**

Stephan Winter[1], Alfred Dixon[2] and Ivan Ingelbrecht[2]; [1]*Head, DSMZ Plant Virus Department, Braunschweig, Germany;* [2]*International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria*

*12.10-12.30* ***Honeysuckle yellow vein mosaic* and *Tobacco leaf curl Japan viruses* with or without DNAβ satellites produce yellow dwarf disease of tomato in Japan**

M. Ikegami, T. Ogawa, T. Ito and P. Sharma; *Department of Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amemiyamachi, Aoba-ku, Sendai, Miyagi 981-8555, Japan*

*12.30-12.50* **Toward identification of grapevine-infecting viruses in vineyards of Iran- *Grapevine fanleaf virus* isolates**

Nemat Sokhandan Bashir, Mohammad Hajizadeh, Shaheen Nournejhad Zarghani, Shahrzad Nikkhah; *Plant Protection Department, University of Tabriz, 29 Bahman Blvd., Tabriz, Iran*

*12.50-13.10* **Characterization of a monopartite recombinant begomovirus and satellite DNAβ associated with yellow vein mosaic disease of mesta crop in India**

Anirban Roy, Raju Ghosh, Sujay Paul, Subha Das, Paramita Palit, Sanchalika Achariya, Javid Iqbal Mir and Subrata Kumar Ghosh; *Plant Virus Laboratory and Biotechnology Unit, Division of Crop Protection, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata – 700 120, West Bengal, India*

Poster Session IV (18 October 2007)

PP-4_99 **Gene silencing suppressor ability of *Tomato leaf curl virus* AC2**

Yogita Deshmukh, Meera Kurulekar, Lenin Kannaiyan, Ravi Kankanallu, Usha Zehr, Rajendra Marathe, Radha Anandalakshmi; *Mahyco Research Center, Jalna-Aurangabad Road, Dawalwadi, Jalna 431203, Maharashtra, India*

PP-4_100 **Non-translatable coat protein of PRSV-P confers resistance in transgenic papaya cultivar Solo**

K.N. Chandrashekara[1], K. Jagadish[1], D.P. Prakash[1], C.M. Kalleshwaraswamy[2], N.K. Krishna Kumar[2] and Akella Vani[1]; [1]*Division of Biotechnology;* [2]*Division of Entomology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore-560 089, Karnataka, India*

PP-4_101 **Cloning and sequencing of complete RNA 3 genome for molecular identification of *Cucumber mosaic virus* causing shoestring on tomato in India**
D. Pratap, S. Kumar and S.K. Raj; *Plant Molecular Virology Group, Centre for Plant Molecular Biology, National Botanical Research Institute, Rana Pratap Marg, Lucknow (U.P.) India*

PP-4_102 **Research on BYDV in Uzbekistan**
Z.N. Kadirova, G.M. Umarova, T.H. Mahmudov, A.Y. Rasulova, O.A. Kadirov and A.H. Vakhabov; *Institute of Genetics and Experimental Biology of Plants, Academy Science of Uzbekistan, 702151 Tashkent, Uzbekistan*

PP-4_103 ***Pepino mosaic virus*: variability in strains**
**R.A.A. van der Vlugt** and Robert van der Meer; *Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands*

PP-4_104 **Molecular variability in the non-structural (NSs) gene of the *Peanut bud necrosis virus* Isolates from India**
P. Sudarsana[1], Prem Rajagopalan[1], Suresh Kunkalikar[1], Usha B. Zehr[1], R.A. Naidu[1] and K.S. Ravi[1]; [1]*Mahyco Research Center, Dawalwadi, Post Box no 76, Jalna-Aurangabad Road, Maharashtra – 431 203 , India;* [1]*Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350*

PP-4_105 **Medicinal mushrooms – a novel source for ribosome inactivating proteins (RIPs) in viral disease management**
R. Radhajeyalakshmi; *Coconut Research Station, TNAU, Veppankulam, Tamil Nadu, India*

PP-4_106 **Cloning and sequencing of coat protein gene of *Sri Lankan Cassava mosaic virus* from Tamil Nadu, India**
R. Padhmavathi[1], R. Rabindran[1], R. Velazhahan[1] and J. S. Kennedy[2]; [1]*Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University (TNAU), Coimbatore, India;* [2]*Department of Agricultural Entomology, Centre for Plant Protection Studies, TNAU, Coimbatore, India*

PP-4_107 **Molecular characterization of *Tobacco streak virus* (TSV) isolates of three economically important crops from Andhra Pradesh.**
B. Sarovar, M. Sreenivasulu and D.V.R. Sai Gopal; *Department of Virology, Sri Venkateswara University, Tirupati-517 502, Andhra Pradesh, India*

PP-4_108 **Molecular cloning and sequencing of coat protein gene of *Banana bract mosaic virus* – Kerala – 1 isolate (BBrMV-KE).**
Lakshmi Unnithan[1], P. Narayanaswamy[1], R. Selvaraj[2], M. Krishna Reddy[3] and V. Balasubramaniam[2]; [1]*Department of Horticulture, Plant Molecular Biology Lab, University of Agricultural Sciences, GKVK, Bangalore,;* [2]*Virology Lab, NRCB, Tiruchirapalli, Tamil Nadu, India;* [3]*Virology Lab, IIHR, Karnataka, India*

PP-4_109 **Strainal variation of rice tungro virus disease in India**
D. Krishnaveni, C.N. Neeraja. G.S. Laha, C.S. Reddy and K. Muralidharan; *Directorate of Rice Research, Rajendranagar, Hyderabad 500 030 AP, India*

PP-4_110 **Rice plant growth promotion and induced systemic resistance against *Rice stripe tenuivirus* by a selected *PGPR, Bacillus amyloliquefaciens* EXTN-1**
Jin-Woo Park[1], Key-Woon Lee[2] and Kyungseok Park[3]; [1]*Research Policy Planning Division, Research & Management Bureau, RDA, Suwon, Korea;* [2]*Department of Agricultural Biology, Kyungpook National University, Daegu, Korea;* [3]*Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon, Korea*

PP-4_111 **Effectiveness of known resistance genes to a *rym5* resistance breaking German BaMMV strain**
A. Habekuß[1], T. Kühne[2], I. Krämer[1], F. Rabenstein[2], F. Ehrig[2], B. Ruge-Wehling[3], F. Ordon[1]; [1]*Federal Centre for Breeding Research on Cultivated Plants, Institute of Epidemiology and Resistance Resources,* [2]*Institute of Resistance Research and Pathogen Diagnostics, Erwin-Baur-Straße 27, D-06484 Quedlinburg;* [3]*Institute of Agricultural Crops, Rudolf-Schick-Platz 3a, D-18190 Groß Lüsewitz, Germany*

PP-4_112 **PRSV-helper component proteinase: more than a RNAi suppressor**
S.K. Mangrauthia[1,2], Suneha Upadhyay[2], R.K.Jain[1] and Shelly Praveen[1]; [1]*Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India;* [2]*Division of Biochemistry, Indian Agricultural Research Institute, New Delhi-110 012, India*

PP-4_113 **Complete nucleotide sequence of *Papaya ringspot virus 'P'* isolate from India**
B. Parameswari[1], S.K. Mangrauthia[2], Shelly Praveen[1] and R. K. Jain[1]; [1]*Unit of Plant Virology, Division of Plant Pathology;* [2]*Division of Biochemistry, Indian Agricultural Research Institute, New Delhi – 110012, India*

PP-4_114 **Expression of *Cucumber mosaic virus* (CMV) coat protein gene (*Gladiolus isolate*) in tobacco**
Vimal kumar Dubey and Aminuddin; *Molecular Virology Laboratory, National Botanical Research Institute, Lucknow, India*

PP-4_115 **Cloning and expression of coat protein gene of *Sweet potato feathery mottle virus* in *E. coli***
Vinayaka Hegde, Ganga Prasanth, T. Makeshkumar, M.L. Jeeva and S. Edison; *Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, Kerala, India*

PP-4_116 **Detection and characterization of a begomovirus associated with leaf curl disease of ornamental croton (*Codieum variegatum*)**
K.S. Shankarappa[1], K.T. Rangaswamy[1], Y.S. Mahesh[1] and M.N. Maruthi[2]; [1]*Dept. of Plant Pathology, University of Agricultural Sciences, Bangalore 560 065, India;* [2]*Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK*

PP-4_117 **PRSV infection on chimeric transgenic (T0) papaya scores tissue for gene integration**
A. Vani, K.N. Chandrashekara, D.P. Prakash and K. Jagadish; *Indian Institute of Horticulture Research (IIHR),Hessaraghatta Lake Post, Bangalore - 560 089, India*

PP-4_118 **Aphid vectors and transmission of *Potato virus* Y strains**
M. Verbeek[1], P.G.M. Piron[1], A.M. Dullemans[1], G. van den Bovenkamp[2] and R.A.A. van der Vlugt[1]; [1]*Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands;* [2]*Nederlandse Algemene Keuringsdienst (NAK), P.O. Box 1115, 8300 BC Emmeloord, The Netherlands*

# *Abstracts of Oral Presentations*

# Session – I: Inaugural Presentation

Keynote presentation

## OP-01: Plant viruses at the ancient ecosystem - recent agroecosystem interface

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The world is undergoing a period of climate change accompanied by rapid expansion in human activity. Both of these factors are impacting on plants, vectors and viruses causing increasing instability within virus-plant pathosystems. This pathosystem instability has major implications regarding mans' ability to achieve effective control of plant virus epidemics that seriously diminish food and fibre production. It also makes this a very interesting era in which to study the changing dynamics of viral epidemiology and evolution in different parts of the world. An important component of such studies involves revealing what is happening with viruses within wild plant populations and cultivated plants at the interface between indigenous vegetation and cultivated areas. This is because research at this interface can provide critical information not only on the potential threats posed to biodiversity and introduced cultivated species by new encounters between viruses and potential host plants which have never come into contact previously, but also on virus evolution and adaptation to rapidly changing conditions. Because of its isolation and the absence of any plant cultivation there before 1829, the Southwest Australian Floristic Region represents an interface between an ancient ecosystem and a recent agroecosystem that provides a unique opportunity to investigate encounters where the virus recipient is an introduced plant and the donor a native plant and *vice-versa*. This presentation will use examples drawn from recent epidemiological and phylogenetic research with viruses in introduced and indigenous plants within this region and elsewhere that illustrate scenarios where newly introduced viruses increasingly damage indigenous plants and where indigenous viruses damage newly introduced cultivated plants. It will also give examples from this region and elsewhere where previously introduced cultivated plants are being damaged by recently introduced viruses. The examples provided will demonstrate how human activities increasingly facilitate damaging new encounters between plants and viruses worldwide. The presentation will also emphasize the kinds of new approaches required if we are to achieve effective control of major plant virus epidemics under rapidly changing world conditions.

# Session – II: Epidemiology and Evolution

Keynote presentation

## OP-02: Microevolutionary dynamics of *Rice yellow mottle virus*: studies at the interface of epidemiology and evolution

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*Rice yellow mottle virus* (RYMV) causes a major rice disease in Africa. First observed in 1966 in Kenya, RYMV is now reported in all countries where rice is cultivated in Africa. RYMV is transmitted at distance by coleopterous and vertebrate vectors, and propagated by contact during cultural practices. The host range is restricted to wild and cultivated rice species. High resistance, mediated by a translation initiation factor, offered the main possibility of control of the disease. Recent molecular analyses complemented the in-depth epidemiological studies on the propagation of RYMV. Isolates of each country where the disease was observed were sequenced. The spatial structure of the diversity, the relationships between geographic and genetic distances and phylogeny suggested a diversification of the virus in Eastern Africa followed by a propagation from East to West across Africa. Infection of islands dozens and even hundreds of kilometres away from mainland Africa showed the possibility of occasional long distance movements. The importance of rice landscape, especially as corridors along the main rivers, in the virus dispersal was underlined. Although RYMV diversity is mostly explained by the geography and its genome is under a high selection pressure, a few sites are under diversifying selection, some of them adjacent to sites experimentally involved in virulence. The current studies are focussed on the tempo of evolution of RYMV. The rate of nucleotide substitution of RYMV (per site and per year) was estimated by Bayesian inference from sequences of a large collection of isolates collected over the epidemiological history of RYMV (40 years). This substitution rate, the first for a plant virus, was compared to a group of animal RNA viruses. Then, it was used to calibrate the phylogenetic tree of RYMV. The dates of appearance of the virus in different regions in Africa were then determined under the hypothesis of relaxed molecular clock. The speed of dispersal of RYMV was estimated. The demography in different parts of Africa was assessed by coalescent analyses. These estimates were compared to epidemiological records of RYMV, to the history of rice in Africa and to the various dispersal modes of the virus in order to identify the key factors involved in the emergence of the disease. These studies, at the interface of evolution and epidemiology, should bring critical information on the ecology of RYMV, and on its ability to overcome resistance, information not accessible by 'classical' epidemiology and plant breeding studies.

Keynote presentation

## OP-03: Plant virus epidemics

**J.M. Thresh**

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Many virus diseases have been described, but relatively few become prevalent and cause serious losses. Inevitably, the diseases that cause damaging epidemics have received a disproportionate amount of attention from researchers and feature prominently in the Plant Pathology literature. This has long been apparent and examples will be given of epidemics that have occurred at various times and places in the 19th, 20th and 21st centuries. A notable feature of any such listing is the great diversity of epidemics. This is apparent in terms of:-

- The many different crops affected, which range from short-lived annuals to woody perennials and include crops usually grown from seed and others from vegetative propagules.
- The regions and agroecologies in which epidemics occur, including tropical, sub-tropical and temperate areas.
- The viruses responsible which include members of many different taxonomic groups.
- The vectors involved. These include soil-inhabiting fungi, eriophyid mites and insects of several different taxonomic groups (aphids, leafhoppers, planthoppers, thrips, mealybugs, whiteflies).

It is also apparent that there is no single underlying cause of epidemics. Some are associated with particular weather conditions, or with a change in agronomic practices, or with the introduction of a new crop or particularly vulnerable cultivar. Other epidemics can be attributed to the introduction of a new virus or virus strain, or with a new vector species or biotype. Whatever the underlying cause, epidemics can be regarded as notable and sometimes catastrophic events but ones that occur infrequently and are seldom sustained. This implies that there are usually powerful constraints to the otherwise unrestricted spread of disease. On this view epidemics are seen as ecological perturbations of the equilibria established between hosts and their pathogens. Furthermore, control measures can be viewed as a means of restoring the equilibrium and thereby safeguarding food security and crop production. The scope and validity of this concept merits further scrutiny and debate if plant virologists are to contribute effectively in attempts to increase the production and productivity of food and other crops. Moreover, they must do so using sustainable and environmentally sound approaches to disease control and despite the decreasing area of land available and a decline in the rural labour force due to urbanization and the ravages of HIV/AIDS.

Keynote presentation

## OP-04: Thrips-transmitted *Iris yellow spot tospovirus* epidemics in the US: progress and challenges in unraveling the epidemiological factors underlying the disease outbreaks in onion seed and bulb crops

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Outbreaks of *Iris yellow spot tospovirus* (IYSV) continue to be a constraint to onion production in several states in the USA. IYSV is transmitted by onion thrips (*Thrips tabaci* Lindeman). Control options to reduce the disease impact are limited. At present, growers are advised to avoid crop stress related to soil fertility, irrigation, and sanitation. Lack of resistance to IYSV in commercial onion cultivars combined with prevalence of high vector populations due to limited thrips control options and the availability of abundant virus inoculum could be leading to the severe disease outbreaks observed in recent years. Molecular studies of IYSV populations based on the nucleocapsid protein sequences showed that there are at least two distinct populations in the US. The epidemiological factors that are contributing to the disease outbreaks have been the focus of several studies. Thrips vectors play a critical role in the outbreaks as there is no evidence of seed transmission. Volunteer onions were found to be infected with IYSV and could be serving as virus reservoirs. In some onion producing regions of the country, the overlapping seasons of the annual bulb and the biannual seed crops provide the green bridge for both the virus and the thrips vector. The role of alternate hosts in IYSV epidemiology is not fully known. In a recent study, several common weeds and cultivated crops, in and around onion bulb and seed fields with a history of IYSV in Idaho and Washington, were sampled from July to October, 2006. Although showing no symptoms, the following weeds were confirmed positive for IYSV by ELISA and RT-PCR: redroot pigweed (*Amaranthus retroflexus*), puncturevine (*Tribulus terrestris*), kochia (*Kochia scoparia*), prickly lettuce (*Lactuca serriola*), and lambsquarters (*Chenopodium album*). This information can potentially be useful for developing targeted cultural and crop management tactics aimed at reducing disease incidence in onion crops. To examine the role of thrips vectors in IYSV epidemiology, an ELISA-based assay is being developed to determine the seasonal dynamics of viruliferous thrips populations. For this, antiserum to NSs, a non-structural protein of IYSV, was produced. The NSs gene was cloned and the protein was expressed in *E. coli*. Purified protein was used to produce polyclonal antiserum. The antiserum reacted with the purified NSs protein, IYSV-infected onion tissue, and not with healthy tissue, *E. coli*-expressed IYSV nucleocapsid protein, TSWV- or INSV-infected tissue. The antiserum is being used to develop an ELISA-based rapid test to determine seasonal dynamics of IYSV transmitters.

# Session – III: Emerging Viruses

Keynote presentation

## OP-05: Evidence of novel viruses by analysis of nucleic acids in virus-like particle fractions from *Ambrosia psilostachya*

**Ulrich Melcher**[1], Vijay Muthukumar[1], Michael J. Palmer[1], Graham Wiley[2] and Bruce Roe[2]

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One challenge in deciding what pathogen caused a disease outbreak is determining what the likely culprits are. Knowledge of what viruses are already present in an affected ecosystem should be useful in assembling a list of possible suspects. The characterization of nucleic acids in a virus-like particle (VLP) fraction of test plants has the potential to provide such information. We developed and tested a procedure that provides such characterization. The procedure isolates VLP from homogenates of plant tissue by differential centrifugation. Nucleic acid in the VLP fraction is released after DNase I treatment by digestion with proteinase K, followed by phenol extraction and ethanol precipitation. The nucleic acid (VNA) is randomly amplified and the products are cloned and sequenced. As a test of the efficiency of the procedure we targeted *Ambrosia psilostachya,* western ragweed, plants growing at the Tallgrass Prairie Preserve of northeastern Oklahoma. Amplifiable nucleic acid was found in seven of twelve specimens and sequences were characterized from four of them. Evidence was found of viruses belonging to three families of viruses (*Caulimoviridae, Flexiviridae, Closteroviridae*) and of one belonging to an unclassified genus, *Endornavirus.* Multiple viral species were found in three of the four specimens and varied in amount among recovered sequences from less than 1% to 37%. None of the sequences were derived from viruses whose sequences have been previously reported. Thus, the virus-like particle-viral nucleic acid method is a useful tool in expanding our knowledge of the universe of viruses and in cataloguing viruses present in an ecosystem of interest. It is being applied to survey viruses present in the Tallgrass Prairie Preserve.

## OP-06: Pospiviroid infections in ornamental plants and their potential risks for vegetable crops

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Six pospiviroids have been reported to cause serious infections in tomato i.e., *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* CLVd), *Tomato apical stunt viroid* TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd) and *Potato spindle tuber viroid* (PSTVd). In most cases the origin of these infections remained unknown. Since CLVd only had been reported from symptomlessly infected plants of three ornamental plant species, the question arose whether ornamentals might function as viroid sources. Therefore, vegetatively propagated ornamental plants were tested for pospiviroids over the last few years. In this way many symptomless pospiviroid infections were detected i.e., CEVd in *Verbena* sp. and *Solanum jasminoides*, *Chrysanthemum stunt viroid* (CSVd) in *S. jasminoides*, TASVd in *Cestrum* sp. and *S. jasminoides*, TCDVd in *Brugmansia sanguinea* and *Petunia* sp. and PSTVd in *Brugmansia* spp., *Datura* sp., *S. jasminoides* and *Streptosolen jamesonii*. All identifications were based on sequence analyses of full-length viroid genomes obtained after RT-PCR with (semi-) universal pospiviroid primers. Trading of ornamental plants may lead to unnoticed viroid spread, as the viroid infected ornamental plants did not show any symptoms. Moreover, some viroid-host plant combinations concerned a large number of plants; for example, the number of PSTVd-infected plants of *S. jasminoides* exceeds 100,000 by far. Viroid spread by trading was shown by testing imported plants. In this way, TCDVd-infected plants of *Petunia* were intercepted in imports from Japan and the USA, and PSTVd-infected plants of *S. jasminoides* were intercepted in imports from Israel and Kenya. On the other hand, testing of symptomless plants of *Brugmansia* spp. and *S. jasminoides* revealed that PSTVd-infected plants of these species very likely were exported from the Netherlands until recently. In our tests many pospiviroid infected ornamental plants have been found. However, their role in the epidemiology of pospiviroids still remains unsolved: further studies are needed to unravel the ways by which viroids might be spread from ornamentals to vegetable crops like tomato and potato. Potential ways of spread by humans and insects will be discussed.

## OP-07: Tomato torrado virus and Tomato marchitez virus, new plant picorna-like viruses infecting tomato

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Tomato plants grown in the field in Murcia (Spain) in 2003 showed symptoms that could not be attributed to any known virus. The first systemic symptoms consisted of necrotic spots at the top of the plant, starting at the base of the leaflets. Necrotic spots expanded and were surrounded by a light-green or yellow area. Plants with severe pronounced necrotic spots on the leaves and obvious growth reduction were found. Locally the disease became known as 'torrado' (meaning roasted), which refers to the burn-like symptoms on the leaves. Electron microscopic investigations revealed the presence of spherical viral particles of approximately 28 nm in diameter in infected leaf tissue. A purification method was developed for the unknown virus and after density gradient centrifugation two virus bands were observed. PAGE showed that virus particles from both bands consisted of three capsid proteins of approximately 35, 26 and 23 kDa. The viral genome consists of two RNA molecules of approximately 8 (RNA1) and 5.5 kb (RNA2). Both RNA species were subjected to cDNA synthesis and cloning. Full nucleotide sequences were determined from cloned cDNA and PCR-generated fragments. Protein sequences of the three capsid proteins mapped them on RNA2. Phylogenetic analyses with the acquired sequence data clearly showed that this virus was not a member of any known plant virus genus. Mechanical inoculation of the isolated virus on tomato resulted in the typical 'torrado' symptoms. The name Tomato torrado virus (ToTV) was proposed for this new virus.

In 2005 it was found that tomato plants from the state of Sinaloa in Mexico, showed symptoms that were remarkably similar to those caused by ToTV; necrotic spots on the leaves, beginning at the base of the leaflets; and dark necrotic rings and patterns on fruit. In Mexico this new disease of tomatoes was commonly referred to as "marchitez", meaning withered or wilted, but an association with the Spanish disease caused by ToTV was never contemplated. Research conducted in the way as performed for ToTV described above showed that the material was infected with a very similar virus, resembling ToTV in both morphology (isometric particles of 28 nm in diameter), number and molecular sizes of coat proteins (35, 26 and 24 kDA) and the number of viral RNA's (two, with sizes of approximately 7.5 and 5.5 kb). Nucleotide sequencing of the full genome of this virus furthermore revealed a clear relationship with ToTV. Back inoculations of the isolated virus to tomato resulted in the typical symptoms as seen in the field. It could be concluded that the causal agent of marchitez disease was related to but distinct from ToTV and it was proposed to name it Tomato marchitez virus (ToMarV).

ToTV and ToMarV are now proposed as members of the new plant virus genus *Torradovirus*, of which ToTV is the type member.

## OP-08: Current status of tospoviruses infecting vegetable crops in India

**K.S. Ravi[1]**, Suresh Kunkalikar[1], M. Bhanupriya[1], P. Sudarsana[1], Prem Rajagopalan[1], Usha B. Zehr[1] and R.A. Naidu[2]

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India is the second largest producer of vegetables in the world, accounting for about 15% of global production. Among various biotic agents, plant viruses play a major role in yield reduction in several vegetables. In recent years, tospoviruses viz., *Peanut bud necrosis virus* (PBNV), *Watermelon bud necrosis virus* (WBNV), and *Iris yellow spot virus* (IYSV), have been found widely prevalent causing significant yield loss in the country. During our surveys it has been observed that the PBNV is found to infect a number of vegetable crops viz., tomato, chili peppers, carrot, egg plant, and various legumes; WBNV in watermelon and muskmelons and IYSV in seed and bulb onion. Polyclonal antibodies raised against PBNV nucleocapsid detected both PBNV and WBNV but not IYSV in direct antigen coating ELISA. In order to study molecular diversity, the N gene sequences of PBNV, WBNV and IYSV were amplified by RT-PCR and the amplicons cloned and sequenced. A comparative nucleotide sequences analysis showed more than 94.9% identity among 67 PBNV isolates, 91.30% identity among 26 WBNV isolates and 95.1% identity among 32 IYSV isolates. Our recent studies have identified the occurrence of *Capsicum chlorosis virus* (CaCV) for the first time in India. The N gene sequence of CaCV showed greater identity with corresponding sequence of CaCV isolate from Australia than those reported from China and Thailand.

# Session – IV: Viruses of Cereal Crops and Soil-Borne Viruses

Keynote presentation

## OP-09: Thirty years of research on soil-borne viruses in cereals - the past and the present in Europe

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In the USA and Japan, diseases of wheat and barley caused by soil-borne viruses have been known for more than 65 years. A first report of soil-borne virus in Europe was given only in 1966, when Canova described the infection of wheat plants in Italy by a virus that is now called *Soil-borne cereal mosaic virus* (SBCMV). Two years later *Oat mosaic virus* was detected in England and after another ten years occurrence of *Oat golden stripe virus* and *Wheat spindle streak mosaic virus* was described in England and France, respectively. First report on the barley infecting yellow mosaic viruses came from Germany in 1978 and only recently in 2003 *Soil-borne wheat mosaic virus* (SBWMV) was detected also in Europe. All these viruses are naturally transmitted by *Polymyxa graminis*. Due to its stable resting spores that keep the incorporated viruses infectious over many years, large cropping areas in Europe became heavily infested in the past. Because use of resistant cultivars is the only way to prevent severe yield losses both research and breeding work have been performed in several countries for more than 30 years now. From the virological point of view main focus in Europe was given to *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV), probably because SBCMV up to 1999 was assumed to be just a European variant of the American SBWMV rather than a definite species. Meanwhile numerous wheat cultivars expressing strong resistance to SBCMV have been developed and the genetic factor for this translocation resistance was recently mapped on chromosome 5DL. Although SBCMV can display considerable genetic diversity possible correlations with biological properties have not been discovered so far. Resistant varieties of rye are still not available, but first individual plants with weak susceptibility to SBCMV could be selected from genetic resources. In case of barley screening programs up to date yielded 16 genes conferring resistance to one or both of the yellow mosaic viruses. The first gene used in a broad spectrum of varieties was the recessive *rym4,* located on long arm of chromosome 3. Recently it has been identified as the eucaryotic translation initiation factor eIF4E. The pathotype BaYMV 2 overcomes this *rym4*-mediated resistance, which obviously results just from single amino acid exchange (Lys to Asn or His) in the VPg. As assessment of many barley genotypes revealed subtle changes in the surface configuration of eIF4E enable or prevent its specific interaction with the VPg molecule leading to susceptibility or resistance. In reaction to BaYMV 2 the *rym5* gene that is allelic to *rym4* was introduced into breeding programs. The first variety containing *rym5* ('Tokyo') became infected by a new pathotype of BaMMV in France and Germany shortly after registration. Analyses of the isolates in the VPg coding region again revealed substitutions both on nucleotide and deduced amino acid levels. To make resistance to BaYMV and BaMMV more durable research programs are in progress to pyramidize different genes in individual genotypes. Despite 30 years of research almost nothing is still known about the specific interactions between the viruses and *P. graminis.* The CP-RT protein of furoviruses was experimentally shown to be directly involved in the transmission process. For bymoviruses P2 is accepted to play a similar role, but the experimental proof is still lacking.

Keynote presentation

## OP-10: Importance of seed and soil-borne transmission in the spread of pecluviruses

**C. Bragard[1]**, B. Dieryck[1], G. Otto[1], A. Legrève[1] and P. Delfosse[2*]

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*Indian peanut clump virus* (IPCV) and *Peanut clump virus* (PCV) are both pecluviruses causing significant losses in crops of groundnut. Both such viruses are seed- and soil-borne, transmitted by the root endoparasite plasmodiophorid vector *Polymyxa graminis*. Wide surveys for the presence of both viruses in either West Africa (Burkina Faso, Mali, Niger, Senegal) or in the Indian sub-continent (India, Pakistan) reveal the presence of clonal and diverse populations of both viruses, linked to the cropping systems in use. The conserved nature of the 3′ end UTR region, as well as the diversity of known viral strains for both viral RNA-1 polymerase and p15 suppressor of gene silencing, as well as on RNA-2 will be stressed. The substantial differences among amino acid composition of pecluvirus coat proteins hamper the serological detection of viruses and asymptomatic association with numerous monocotyledonous and dicotyledonous plants complicates diagnosis. These problems in virus detection triggered the design of a broad spectrum RT-PCR method for virus systematic detection. The lack of control measures also emphasizes the need for a better understanding of the virus and its vector epidemiology. The role of alternative hosts, disease such as sugarcane red leaf mottle, the importance of seed transmission in such cereals as finger, pearl and foxtail millet and wheat, and the influence of the crop rotation and of climatic conditions as well as irrigation will be discussed. Present knowledge on pecluviruses stresses the risks of disease spreading by seed exchanges and soil movements, as well as the need for a disease management taking the different cropping systems into account.

## OP-11: Cereal viruses in the Czech Republic: distribution, detection, genetic diversities and resistance

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Barley yellow dwarf (BYD) and wheat dwarf (WD) diseases belong to the economically most important diseases in cereal crops in the Czech Republic. The incidence of these viruses occurred even epidemically in some years during last decade. BYD disease is caused by *Barley yellow dwarf virus* (BYDV), group of positive single stranded RNA viruses of the family *Luteoviridae*, transmitted by aphids. The causal agent of WD disease is *Wheat dwarf virus* (WDV), ssDNA (Mastrevirus), transmitted by leafhopper *Psammotettix alienus*. During the last four years the incidents of WDV were recorded frequently and often in epidemic proportions in some regions, causing significant economical losses. The mixed infections of these viruses were also recorded very often. Besides BYDV and WDV, the occurrence of other cereal viruses like *Wheat streak mosaic virus* (WSMV), *Brome mosaic virus* (BMV) and *Oat blue dwarf virus* (OBDV) were also reported earlier. RT-PCR based detection targeting coat protein (CP) gene followed by restriction was used for the detection of BYDV. A laboratory isolate of BYDV PAV Blatno85 and several field isolates were subjected to CP gene sequencing for the analysis of genetic diversity of BYDV. The CP gene sequences and restriction profiles of *Sty* I and *Hpa* II of the PCR products proved the occurrence of at least three BYDV strain, PAV, MAV and PAS among the field isolates of BYDV. Similarly, WDV laboratory wheat isolate, WDV-Pkr, barley isolate WDV-Jkr and several field isolates were subjected to analysis. PCR based detection of WDV CP gene and following restriction and sequence analysis have confirmed occurrence of both wheat and barley strain in the field samples. PCR assay using strain specific primers was elaborated to identify wheat and barley strains of WDV. A duplex RT-PCR has been developed for the identification of BYDV and WDV in a single reaction. Resistance program of cereal crops aiming to achieve natural source of resistance against BYDV and WDV has been performed for the last 15 years in our laboratories. Different resistance traits, especially of the winter barley and wheat cultivars and breeding lines have been evaluated. A series of winter wheat/barley cultivars and several species of *Aegilops* have previously been tested and any sources of resistance to WDV were identified. However, some wheat cultivars (*e.g.* Banquet, Svitava) shown field tolerance and in green house experiment reacted as moderately susceptible to WDV infection. Evaluation of resistance of cereal materials to BYDV conducted for long term field experiments has shown several significant sources of resistance, which confer high level of protection against the BYDV infection. The cereal materials containing resistance gene such as Yd2 (barley), Bdv1, and Bdv2 (wheat) in general have shown promising results.

## OP-12: Evaluation of the appearance of different genotypes of *Wheat dwarf virus* in Germany

**J. Schubert[1],** A. Habekuß[2], K. Katzmeyer[3] and Holger Jeske[3]

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During the last decade in Germany a growing appearance of *Wheat dwarf virus* (WDV) was noticed. Probably, this is also a result of changing climatically conditions. Except in Europe the virus was also detected in North Africa and throughout China. The virus is transmitted by the plant leafhopper *Psammotettix alienus* which is spread worldwide. Two genotypes of the virus were described and sequenced. They were designated as barley and wheat genotypes. Usually, the virus is detected by means of ELISA or PCR. The latter also enables differentiation of both genotypes using specific primers. Recently, a new method for DNA amplification has been described named Rolling Circle Amplification (RCA). It utilizes DNA polymerase Phi29 amplifying circular molecules. Using this technique in combination with an RFLP analysis we surveyed appearance of different genotypes of the virus in Germany. By means of this method we identified a new genotype of the virus in oat. It is not transmitted to other cereals than oat by *P. alienus*. The complete genome of this genotype was cloned and sequenced. Some of the full length restriction fragment sequences of wheat and barley genotypes collected in 2006 and 2007 were cloned too. It appeared that both genotypes formed rather invariable haplotypes. We never observed recombinant variants between any of the three known genotypes. As the sequence homology for all three genotypes is lower than 80% we suggest to refer them as different viruses – *Wheat dwarf virus*, *Barley dwarf virus* and *Oat dwarf virus* forming the complex of Cereal dwarf viruses. In the field survey a mixed infection of different genotypes was not observed so far. In some cases we found wheat to be infected with *Barley dwarf virus*. Under greenhouse conditions we were unable to infect barley with *Wheat dwarf virus* or wheat with *Barley dwarf virus*. Several methods have been tested to simplify testing procedure. Detection was possible from freeze dried tissue after half year storage at 4°C with a simple buffer system. Twenty-five mg of plant material were sufficient for repeated virus analysis and cloning experiments. The advantage of RCA lies in its simplicity, extreme sensitivity and ability to detect unknown genotypes of this virus complex.

# Session – V: Biosecurity and Modelling

Keynote presentation

## OP-13: The role of plant biosecurity in preventing and controlling emerging plant virus disease epidemics

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The emergence of a global community and an increase in plant virus discovery in the last 15 years has increased the requirement for countries and regions to protect their farming systems from these exotic pests. In a recent report plant viruses were identified as the cause of 47% of the emerging infectious diseases of plants that were recorded on the Promed database during the period of 1996 – 2002 and a further 4% were attributed to phytoplasmas and it is likely that this trend will continue. Plant biosecurity is defined as a set of measures designed to protect crops from emergency plant pests (EPPs) at national, regional and individual farm levels. In addressing plant biosecurity issues countries need to comply with its international obligations as defined by the WTO Agreement on the application of Sanitary and Phytosanitary measures. More specifically there are international guidelines and standards outlined under the International Plant Protection Convention that stipulate that a pest/pathogen is "known not to occur" in a given geographic region. In this context a number of research strategies have been initiated over the last decade to enhance our biosecurity capacity at the pre-border, border and post-border frontiers. In preparation for emerging plant virus disease epidemics, diagnostic manuals for economically important plant viruses that threaten local industries (e.g. *Plum pox virus*) have been developed and validated under local conditions. Contingency plans have also been documented that provide guidelines to stakeholders on diagnostics, surveillance, survey strategies, disease epidemiology and pest risk analysis. Reference collections containing validated positive controls have been expanded to support a wide range of biosecurity sciences. Research has been conducted to introduce high throughput diagnostic capabilities and the design and development of advanced molecular techniques to detect virus families. These diagnostic tools can be used by post entry quarantine agencies to detect known and unknown plant viral agents. Pre-emptive breeding strategies have also been initiated to protect plant industries if and when key exotic viruses become established in local communities. With the emergence of free trade agreements between trading partners there is a requirement for quality assurance measures of pathogens, including viruses, which are present in both the exporting and importing countries. These measures are required to ensure market access for the exporting country and also minimize the risk of the establishment of a disease epidemic in the importing country.

Keynote presentation

## OP-14: Use of GPS, GIS, and geostatistics to develop pre-plant virus disease prediction models

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Management of bean leaf beetle (*Cerotoma trifucarta* Foster) populations and consequently *Bean pod mottle virus* (BPMV), would be improved if the percent mortality of overwintering bean leaf beetle populations could be accurately predicted before spring planting. The objective of this study was to evaluate the potential for developing a model that will accurately predict (pre-plant) the incidence of BPMV in Iowa counties. We first obtained quantitative information concerning the actual incidence of BPMV in each of Iowa's 99 counties, based upon data obtained in a state-wide soybean disease survey conducted during the 2005, 2006, and 2007 soybean growing seasons. Approximately 10 to 12 soybean fields per county were sampled and tested each season for the presence of BPMV by ELISA (Elkhart, IN). The average incidence of BPMV was then mapped using ArcGIS software and these values were used as the dependent variable (y) to test the predictive ability of percent mortality estimates generated from a model developed by Lam and Pedigo (Envirn. Ento. 29:800-806). The predicted mortality for bean leaf beetles in 2005 and 2006 were also mapped (county level) and compared with county-level maps depicting actual BPMV incidence (based on the state-wide soybean disease survey of ~1,000 soybean fields tested for each growing season). Percent mortality estimates were then used as the independent variable to predict BPMV incidence using linear regression. Regression models relating % mortality (x) versus BPMV incidence (y) indicated that predicted % mortality explained 11.1% and 38.1% of the variation in BPMV incidence for the 2005 and 2006 soybean growing seasons, respectively. In a model proposed by Nutter and Robertson, the number of days that the mean daily temperature was < 0° C explained 47% of the variation in BPMV incidence at the county level for 2006. The addition of other risk factors (i.e. duration of snow cover, snow depth, soil temperatures, etc.) may further increase the accuracy of model predictions.

## OP-15: Role of epidemiology of seed-transmitted viral diseases for developing seed certification standards for grain legumes in India

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Studies on epidemiology have a significant role in management of economically important seed-transmitted viruses which remain infective in seeds as a primary source of inoculum. Even a very low rate of seed transmission of the virus can lead to an epidemic situation in the field due to spread of disease through insect vectors, mainly aphids, and in certain cases through nematodes, beetles and fungi. Efforts have generally not been made to generate, compile and correlate data on epidemiology in a holistic manner so as to develop disease management strategies based on seed certification programmes and resistance as viruses cannot be effectively managed otherwise. Initiatives have been taken to generate epidemiological information for important seed-transmitted viruses of grain legumes viz., *Bean common mosaic virus* (BCMV) and Urdbean leaf crinkle disease (ULCD) of black gram and green gram, *Black eye cowpea mosaic virus* (BlCMV now a strain of BCMV) and *Cowpea aphid-borne mosaic virus* (CABMV) of cowpea, *Soybean mosaic virus* (SMV) of soybean and *Pea seed-borne mosaic virus* (PSbMV) of pea in order to develop a model system of seed certification for some of them. Based on extensive surveys carried out for three years in nine major legume-growing states in India complemented by testing 1842 seed samples collected from diverse agencies from 21 states, a national map on prevalence of seed-transmitted viruses of grain legumes was prepared and studies revealed that the disease incidence varied with the location and the crop variety. The detection and identification of viruses both in leaves and seeds were done by deploying a combination of growing-on test, infectivity assay, electron microscopy, ELISA and RT-PCR. Studies on the samples tested by growing-on test and ELISA testing of leaf samples revealed a seed transmission rate of up to 16% of BlCMV and 28% of CABMV in cowpea; up to 67%, 49% and 60% of BCMV in black gram, green gram and French bean, respectively; up to 55% of PSbMV in pea; up to 52% of SMV in soybean; up to 55% and 6% ULCD in black gram and green gram, respectively. The results gave preliminary indication on number of sites in different states that were found to be free from certain viral diseases. A correlation in viral disease incidence with aphid vector population, and appreciable losses in seed yield were observed. Based on virus spread using a known level of initial seed/ seedling infection, the seed standards for certification against viruses of cowpea and soybean were proposed to be 0.5% and for pea as 2%. ELISA-based diagnostic kits against BlCMV and SMV were prepared to be efficiently utilized for quality control of seeds. The importance of epidemiology need to be realized for management of viral diseases in an integrated pest management programme and also for generating information on pest-free areas and for pest risk analysis which is now an obligation in international trade.

## OP-16: A survey of grapevine viruses: epidemiology from an instant picture

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Virus epidemics on woody hosts are slower than on herbaceous ones, according to their longer life span. This way, several years' work is necessary to detect disease progress. However, some clues can be obtained from the spatial distribution of affected vines at a given time. The Rioja wine region in Northern Spain is renowned by its red wines, either young or oak-matured. A survey on the predominant variety Tempranillo was done in winter 2005-2006. Canes from 50-100 plants were taken from each 20 vineyards representative of different altitudes, rootstocks, ages and training systems. They were tested by ELISA for *Grapevine fanleaf virus* (GFLV, *Nepovirus, Comoviridae*), *Grapevine fleck virus* (GFkV, *Maculavirus, Tymoviridae*), *Grapevine virus A* (GVA, *Vitivirus, Flexiviridae*) and *Grapevine leafroll-associated viruses 1, 2* and *3* (GLRaV-1 and -3, *Ampelovirus, Closteroviridae*; GLRaV-2, *Closterovirus*). No GVA was detected. GLRaV-1 was rare. The mildest viruses, GFkV and GLRaV-2, were widespread, affecting most vineyards with a high incidence (over 50%, in some cases). The severe viruses GFLV and GLRaV-3 had an intermediate incidence (up to 30%). No correlations among the infections by the different viruses were found, in spite of GLRaV-1 and 3 sharing coccid vectors. The analyses of variance found no statistical significance of the factors altitude, rootstock, age and training system. GFLV showed an aggregated spatial distribution, which could be indicative of active transmission by its nematode vector. GLRaV-3 was randomly distributed, which could be indicative of initial infection from the plant material, not followed by vector activity. Clonal selection blocks, planted in the late '80s with virus-indexed material on nematode-free ground, were surveyed in winter 2006-2007. They remain virtually free of these viruses, which also favours the idea of a lack of coccid activity and confirms the usefulness of clonal selection programmes in controlling grapevine viruses. GFLV is known to seriously reduce fruit production. The effect of GLRaV-3 is not so severe on yield, but is known to reduce sugar content and colour intensity, while increasing acidity. In microvinifications done from plants affected or not by these viruses, GLRaV-3 was found to decrease final alcohol contend by half a degree and seriously diminish colour intensity. Wine from GFLV-infected plants had no decrease in quality, but yield was poor.

## OP-17: Landscape patterns of aphid vectored viruses of pea in the Palouse region of Idaho and Washington: implications for virus forecasting

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Legume crops in southeastern Washington State and adjoining Idaho, USA are periodically affected by virus diseases. At roughly 5 to 7 year intervals, disease caused by *Pea enation mosaic virus* (PEMV), *Bean leaf roll virus* (BLRV), *Pea streak virus* (PSV), or some combination of these viruses causes severe yield losses and risks of crop failure. Although the virus epidemic years roughly coincide with years of high aphid populations (outbreak years), the association is not consistent, so that in some years, despite relatively high aphid populations, virus risk is low, and vice versa. Moreover, disease can occur sporadically in individual fields in most years. Whether it is economical for producers to treat aphids aggressively with insecticides depends upon whether virus inoculum in these aphids is sufficiently high. In 2007, a study was initiated to determine the viability of monitoring immigrating pea aphids for PEMV, BLRV and PSV in order to assess risk of virus infection in legumes grown in the region. A network of traps covering the region was used to monitor arriving alate pea aphids and assess them for the presence of viruses using a PCR-based method. Temporal and spatial patterns of the influx of aphids and the occurrence of virus in these aphids, and subsequently in the crop, reveal an apparent higher risk for virus to the south and west of the study region. Aphids also were first detected in this part of the region. The patterns are consistent with a hypothesized source of aphids and viruses to the south and east of the legume growing region of southeastern Washington and adjoining Idaho. Although the incidence of viral disease in peas was low in the region in 2007, this year's data potentially provide a baseline for detecting onset of years with greater risk of virus infections.

# Session – VI: Virus-Vector Evolution & Interactions

Keynote presentation

## OP-18: Helper-dependency in vector-transmission: ecological role of a complex process too often adopted by plant viruses

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Nearly all plant viruses need vectors to spread from one host to another. The most widely adopted strategy for virus-vector interaction is "non-circulative" transmission, and the most common vectors are arthropods, especially sap-feeding insects. In contrast to viruses of animals and plants that replicate or circulate within their insect vectors, the pattern of non-circulative transmission is apparently simple: the virus is taken up by a vector feeding on an infected plant, adsorbed somewhere on the cuticular lining of the inner part of the feeding apparatus, and subsequently released to inoculate a new host plant. Nevertheless, molecular studies have unraveled unsuspected levels of complexity in the mechanisms controlling this process. Two molecular viral strategies have been reported: the "capsid strategy" where domains of the coat protein directly recognize unknown retention sites in the vector mouthparts, and the "helper strategy" where a non-structural viral protein, designated helper component (HC), creates a molecular link between viral particles and the vector. Though involving more complex regulations, the helper strategy is by far the most widely adopted by non-circulative viruses, suggesting an adaptive role towards unknown constraints related to vector-transmission. Because HC can complement the vector-transmission of related genetic variants within a viral population (HC-transcomplementation), it has been hypothesized that the helper strategy could alleviate bottleneck-related problems during plant-to-plant transmission, by augmenting the genetic diversity, and thus the effective size of the viral population passing through this step. We have investigated this hypothesis on the example of *Cauliflower mosaic virus* (CaMV), and consistently demonstrated the existence of strong CaMV population bottleneck during aphid transmission. However, the distribution of CaMV genetic variants within the infected plant and within single cells, together with information on the feeding behavior of the aphid vectors, indicate that the viral sample taken up by the vector may not significantly differ whether HC-transcomplementation occurs or not. In another hypothesis, the helper components could regulate the competition, between co-infecting virus species, for a putative ubiquitous receptor in the vector's mouthparts. This alternative hypothesis is now being explored through the search and preliminary characterization of such putative receptor for CaMV within the aphid vector stylets. The future development of this work will investigate the possible use of the same receptor in vectors, by several virus species.

Keynote presentation

## OP-19: Behavioural aspects of virus transmission by Hemipteran insects

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Hemipterans (aphids, whiteflies, leafhoppers and mealybugs among others) are the major vectors of virus diseases comprising more than 80% of insect-transmitted viruses which represents close to 400 virus species within 39 different genera. Virus transmission by hemipterans requires a series of steps including host searching behaviour, probing on superficial leaf tissues, settlement and feeding. Most efforts have concentrated on aphid-transmitted viruses and particular behavioural processes leading to the transmission of non-circulative and circulative viruses have been identified. This paper will review on how viruses and other plant pathogens have adapted to exploit particular feeding habits of their insect vectors to spread from plant to plant. In particular, some phloem-restricted viruses (e.g. Luteoviridae and Begomovirus) use phloem feeders such as aphids or whiteflies to spread after circulation through the insect's hemocoel and excretion by the salivary glands. Others (e.g. Closteroviridae) are also acquired from the phloem but remain attached to the mouthparts of the vector until subsequent feeding on the phloem tissue of a healthy plant. Xylem-restricted bacteria are vectored by some Cicadellidae that penetrate the plant tissue intracellularly to feed in the xylem vessels. Other viruses (e.g. Caulimoviruses) are transmitted after several intracellular stylet punctures before starting phloem salivation or feeding. On the other side, there are viruses (e.g. Potyvirus) that have a very ephemeral interaction with their vector and are transmitted to the epidermis after single intracellular stylet punctures during host searching behaviour and are lost before the vector reaches the vascular tissues. However, little is known about the transmission of viruses by mealybugs, such as *Grape leaf roll virus-3* (GLRaV-3) and other Ampelovirus. Recent studies on stylet activities during plant penetration by aphids have broadened our knowledge on the role of salivation in the transmission of plant viruses and on the localization of putative receptors among the vector´s mouthparts. This presentation will revue the current knowledge on behavioural events related to the transmission and spread of plant viruses by their hemipteran vectors. Furthermore, new information on the behaviour of the citrus mealybug, *Planococcus citri* (Hemiptera: Coccidae), vector of GLRaV-3 and of *Bucephalogonia xanthophis* (berg) (Hemiptera: Cicadellinae) vector of *Xylella fastidiosa* will be presented.

## OP-20: Testing of the vector dependency hypothesis with aphids and whiteflies

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The concept of 'vector dependency' by viruses was proposed as a way to better understand the outcomes of interactions among viruses, vectors and plants from the standpoint of the vector species involved. The fitness of a vector is closely linked to host plant quality, which in turn may be influenced by virus-mediated changes that affect plant nutritional attributes for the vector. Because viruses vary considerably in their relations with vectors, from complete dependence upon a principal vector species for dispersal to complete independence, i.e. a non-vectored virus, the relative dependency of a virus on one or more vector species may be important to understanding outcomes from a vector performance perspective. The vector dependency hypothesis posits that viruses that are more dependent upon vectors will indirectly benefit those vectors through favorable changes in the quality of their host plants. Conversely, less dependent viruses do not have as much at stake from an evolutionary perspective, and therefore are not under as much selection pressure to mediate changes in host plant quality that benefit vector species. In addition, the relative complexity of the virus-vector relationship will likely correspond to degree of dependency as a virus that exhibits a more complex mode of transmission (e.g. circulative) will be more specific in its relationship with a particular vector and therefore more dependent. To test this hypothesis, three potato viruses that vary in their dependency on the aphid vector *Myzus persicae* were tested in potato plants to determine if putative virus-mediated changes in plant quality affected various life history traits of *M. persicae*. The three viruses were potato leafroll (PLRV), transmitted circulatively by its principal vector *M. persicae*; *Potato virus Y* (PVY), transmitted non-persistently by numerous aphid species; and *Potato virus X* (PVX), a non-vectored virus. All life history traits including rate of increase, longevity and fecundity were significantly greater for *M. persicae* on PLRV-infected plants compared to plants infected by either of the other two viruses or the virus-free plants. A second virus-vector combination is presently being investigated to test the robustness of the vector dependency hypothesis. This system consists of *Bemisia tabaci* as the insect vector of the *Cotton leaf crumple virus* (CLCrV) on two different host plants, *Gossypium hirsutum* and *G. thurberi.* Initial tests on CLCrV-infected vs. uninfected upland cotton (*G. hirsutum*) failed to detect any benefit to *B. tabaci* on CLCrV-infected plants. However, the nature of the *G. hirsutum*/CLCrV relationship is a recent one, dating back to the mid-20$^{th}$ century when cotton was first cultivated in the southwestern USA. In contrast, interactions among *G. thurberi*, *B. tabaci* and CLCrV probably date back well before the 20$^{th}$ century as all are indigenous to the southwestern USA. Evidence of this long association is apparent in the mild symptoms that occur in *G. thurberi* relative to the severe symptoms observed in *G. hirsutum*. Tests currently in progress will measure life history traits of *B. tabaci* on virus-infected and virus-free *G. thurberi* as well as relative attractiveness of plants to *B. tabaci* according to virus status.

Keynote presentation

## OP-21: Mixed-viral infections (PVY-PLRV) affect the biology and preference of aphid vectors and consequently the epidemiology of potato viruses

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*Potato virus Y* (Potyviridae: *Potyvirus*) (PVY) and *Potato leafroll virus* (Luteoviridae: *Polerovirus*) (PLRV) are the two most economically important viruses in the potato (*Solanum tuberosum* [L.]) crop in Idaho, the number one potato producing state in the United States. An increased number of plant samples from Idaho's potato fields over the last years has serologically tested positive for both PVY and PLRV via double antibody sandwich enzyme linked-immunosorbent assay (DAS-ELISA). These viruses are efficiently transmitted by the same species of aphid vectors: green peach aphid, *Myzus persicae* (Sulzer) and the potato aphid, *Macrosiphum euphorbiae* (Thomas), (Homoptera: Aphididae). These viruses have opposite effects on the vector behavior; the persistently transmitted virus PLRV attracts and arrests the vectors longer than the non-persistent virus PVY to encourage sustained feeding for its successful transmission. PVY manipulates its vectors by supporting shorter feeding periods (probes) sufficient enough to transmit the virus. It has previously been shown that virus infections influence the quality of plants directly affecting the performance of aphids feeding on these plants. Despite the common occurrence of the combined infection of PVY with PLRV in potato, to the best of our knowledge nobody has examined their interactions and probable synergisms. Probable synergisms if evidenced, could lead to titer changes of one or both viruses in the plant host, which could have a profound effect on the vector biology and behaviour and consequently an effect on the epidemiology of these viruses on potatoes. Therefore, the objective of this study was to determine the consequences of mixed infections in both the potato plant and the insect vectors and how these mixed infections in turn affect the vector dynamics and disease epidemiology. Laboratory studies were conducted to examine the effect of mixed-viral infection on the fecundity and preference of the two most efficient vectors, the *M. persicae* and *M. euphorbiae*. Four treatments (Potato control, $PVY^N$-infected, PLRV-infected and $PVY^N$-PLRV infected) were used in these experiments. These treatments were replicated 10 times and the virus titers quantified and compared by DAS-ELISA to attest the presence or absence of synergism. Life histories of *M. persicae* and *M. euphorbiae* were studied on the above-mentioned treatments using leaf cages. Various parameters like nymphal period, adult longevity, daily fecundity and intrinsic rate of increase were evaluated on all the treatments. To study the effect of mixed infections on the host selection behavior of their aphid vectors settling bioassays with alatae and apterae *M. persicae* and *M. euphorbiae* were conducted on the treatments mentioned above. *Myzus persicae* and *M. euphorbiae* fecundity was significantly higher on mixed-infected plants than on singly-infected plants or non-infected plants. Both winged and wingless *M. persicae* and *M. euphorbiae* preferentially settled on PVY-PLRV infected plants than on singly-infected plants (PVY or PLRV) or non-infected plants. Mixed-viral infected plants with these two heterologous viruses exhibited more severe symptoms than singly-infected plants (PVY or PLRV). Results of this study revealed the intricacies of vector-virus and host plant interactions and will help predicting the vector dynamics and disease epidemiology better. The results of this study will also help formulating and justifying various potato virus and vector control strategies.

## OP-22: Evidences for transmission of *Indian cassava mosaic virus* through *Bemisia tabaci* - cassava biotype

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*Indian cassava mosaic virus* (ICMV) (*Geminiviridae*: *Begomovirus*) is spread primarily through the infected cuttings and secondarily by whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). A cassava adapted haplotype of *B. tabaci*, referred to as cassava biotype and a polyphagous sweet potato reared population (but does not breed on cassava) identified as sweet potato biotype were reported from India. Recent studies have shown that ICMV is transmitted by the cassava biotype of *B. tabaci*, but not by the sweet potato biotypes. This study was initiated to further investigate the ICMV transmission by cassava-biotype population of *B. tabaci*. Virus acquisition access (feeding) period (AAP) of 48 h on ICMV-infected cassava leaves, and 48 h virus inoculation access (feeding) period (IAP) on virus-free cassava seedling leaves were investigated. ICMV was successfully transmitted from cassava to cassava by cassava biotype. A triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) used to detect geminivirus in experimental infected plants confirmed the presence of ICMV. Later, ICMV purified from the experimental cassava plants by sucrose density gradient ultracentrifugation and analyzed by immunosorbent electron microscopy (ISEM) by using specific gold-labeled goat anti-rabbit IgG in which isometric geminate particles of 18-20 nm could be detected. Nested PCR primers designed from the highly conserved coat protein gene of ICMV – Trivandrum isolate was successfully used for detecting ICMV in stylet, salivary gland and digestive tract of single *B. tabaci*- cassava biotype after a 48 h AAP, confirming cassava biotype whitefly as the ICMV vector involved in the virus transmission from cassava to cassava.

## OP-23: Risk of spread and vector relations of *Potato yellow vein virus* in the Andes

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The emergence and management of plant viruses transmitted by insect vectors is an important phytosanitary problem, particularly in developing countries, where plant health systems are not adequately equipped. Key factors responsible for the emergence of new plant diseases include: the intensification of agricultural trade (globalization); changes in cropping systems (*e.g.* crop diversification); and climate change. The best plant disease control strategy is 'exclusion' of the pathogen, which requires the implementation of sensitive plant virus detection methods and production of virus-free planting material. *Potato yellow vein virus* (PYVV) is a threat for potato cultivation in the Andean region because of its wide and rapid dissemination through infected planting material and a ubiquitous insect vector: the whitefly species *Trialeurodes vaporariorum*. PYVV, a tentative *Crinivirus*, has spread in the last 7-10 years throughout the Andean region of Colombia and Ecuador, and is now present in northern Peru. The Tropical Whitefly IPM Project (TWFP) of the CGIAR Systemwide IPM Programme is assessing the risk of the spread of PYVV in the remaining potato growing areas of the Andean region, by using Geographic Information Systems (GIS) technology to map the current distribution of the disease. Mapping data of the incidence of PYVV and its whitefly vector in Peru (from North to South: 22 places in departments of Cajamarca, La Libertad, Ancash, Huanuco, Lima, Arequipa, and Tacna) suggest that the virus could spread to south and east Peru through infected potato seed where the insect vector is already present. So far, the disease has only been detected in Cajamarca, La Libertad, and Ancash. The survey conducted in Ecuador (23 places in the provinces of Tungurahua, Cotopaxi, Carchi, and Ibarra) indicates that the virus is being disseminated through infected potato seed. The virus was present in all provinces visited, but the insect vector was only observed in the provinces of Tungurahua and Ibarra. The survival and dissemination of the insect vector of PYVV is also aided by the presence of other suitable whitefly hosts, such as *Lycopersicon esculentum, Phaseolus vulgaris,* and *Cucurbita pepo*. Thus, the management of PYVV depends on the implementation of sensitive virus detection methods, production of virus-free planting material, and integrated whitefly management practices in the potato-growing areas where *T. vaporariorum* is currently present or can survive. Forecasting the future distribution of the insect vector with the help of GIS technology is critical in predicting the effect of climate change and different IPM practices on the potential spread and impact of PYVV in the Andes.

## OP-24: Reducing the global impact of thrips-transmitted tospoviruses in diverse cropping systems: successes gained and challenges that lie ahead

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Diseases caused by tospoviruses continue to impact the yield and quality of several important field, horticultural, and ornamental crops resulting in serious economic damage. While *Impatiens necrotic spot virus*, *Iris yellow spot virus* (IYSV) and *Tomato spotted wilt virus* (TSWV) continue to be of economic significance in the USA, increased tospovirus diversity is becoming apparent in Asia and South America. The expanding host range and shifting specificities of thrips-tospovirus associations have become more prominent in certain Asian countries such as India. TSWV has been a major constraint to the production of peanut, pepper, tobacco, and tomato in the southeastern US for over a decade. Reducing the impact of spotted wilt was difficult: TSWV has a wide host range that includes numerous weeds, affected crops have overlapping growing seasons, and the two known thrips vectors, western flower thrips (*Frankliniella occidentalis)* and tobacco thrips (*F. fusca*) are well-established and have a wide host range. Crop-specific management tactics had to be developed since certain virus suppression tactics found to be effective for one crop had little or no effect on others. An integrated disease management (IDM) strategy was found to be the most successful. For peanut, the IDM consisted of using resistant varieties in combination with certain cultural practices such as adjusting the planting date, seeding rate and row spacing. Use of resistant varieties and plastic mulches significantly reduced the impact of TSWV in pepper and tomato. Interestingly, in the case of tobacco, use of plant defense activators such as acibenzolar-S-methyl in combination with imidacloprid resulted in a significant reduction of the disease. Thus, experience with spotted wilt highlights the need for development of an IDM strategy for other tospoviruses such as IYSV*, Peanut bud necrosis virus* and *Watermelon bud necrosis virus* that are prevalent in India. Surveys followed by molecular characterization of tospoviruses from various vegetable crops provided important information on the prevalence and genetic diversity of these viruses in India. Identification of risk factors that contribute to the virus outbreaks need to be identified. The role and seasonal dynamics of various thrips vector species, the nature and prevalence of virus strains, breeding for virus resistance or field tolerance using host- or pathogen-derived resistance, and incorporating crop-specific risk mitigation practices are likely to help develop an IDM strategy in reducing the impact of these viruses.

# Session VII: IPVE Committee General Session

## OP-25: The Plant Virus Ecology Research Coordination Network

**Ulrich Melcher**[1], Yan Song[1], Tony Bridgewater[1] and Carolyn Malmström[2]

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Plant virus ecology, an emerging field, examines the ecological roles of plant-associated viruses in managed and unmanaged ecosystems, and investigates the reciprocal influence of ecosystem properties on the distribution and evolution of plant viruses. Viruses are frequently found not only in crops but also in wild plants growing in unmanaged environments. However, virus influence on wild hosts and the nature of virus exchange between managed and unmanaged systems remains poorly understood. One reason for the sparsity of plant virus ecology information is the absence of vigorous interaction between the disciplines of plant virology and ecology. The work reported here has two objectives: 1) to provide ecologists an electronic key for identification of plant viruses; 2) to establish a research coordination network for plant virus ecology. The aim of the electronic key is to facilitate ecologists' identification of potential virus species of interest in their study ecosystems. While adept at investigating complex community interactions in the field, most ecologists have not had the opportunity to become familiar with virus taxonomy and distribution and such a taxonomic tool may help stimulate identification of study systems of joint virological-ecological interest. Currently available information on plant viruses, such as the Descriptions of Plant Viruses and its database and the latest report of the International Committee on the Taxonomy of Viruses is organized in such a way that an investigator wishing to use it must be able to identify a candidate virus before being able to use the resources. To make such information more accessible to ecologists and others less familiar with virus taxonomy, we assembled information from these sources into a MySQL database and made the information accessible through a web interface search page. The Perl-based scripts allow retrieval of a list of viruses matching entered criteria, which can include genome properties, particle shape and size and capsid protein sizes, among others. For the second objective, two of us (UM and CM) solicited and obtained support from the US National Science Foundation for support of a Plant Virus Ecology Research Coordination Network. During the next five years, the network will, among other activities: 1) bring together an initial core of researchers (virological through ecological) currently working on aspects of plant virus ecology in US and international laboratories; 2) through a series of initial annual meetings, workshops, and mediated discussions, evaluate infrastructural needs, prioritize a research agenda, and take steps to expand the emerging community and build long-term support needed for the field; 3) develop a prototype interactive plant virus ecology exhibit and public school outreach programs with a nationally regarded science museum, to engage young people and the general public from diverse backgrounds. Interested individuals are invited to participate.

# Session VIII: Advances in Virus Disease Management

### Keynote presentation

## OP-26: Natural resistance mechanisms to viruses in plants

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During evolution most plants became resistant or tolerant to most viruses (and other diseases); however, the resistance mechanism in most cases poorly understood. One may distinguish between active resistance involving R-genes and signal transduction, and passive resistance, for example lack of components required for viral replication as the plant coded segment of the viral replicase. The following resistance phenomena and mechanisms will be discussed:

- Gene silencing: This is a kind of immune defense mechanism whereby foreign nucleic acids as viruses, transposable elements and transgenes are degraded, involving small interfering double-stranded RNAs (siRNAs). Thus, some of the transgenic tomato plants, expressing the siRNA targeted against the *Tomato yellow leaf curl virus* CP gene, did not show disease symptoms 7-weeks post-inoculation with the virus. This mechanism is probably responsible in many cases for the resistance of non-hosts. Some viral proteins act as suppressors of gene silencing.
- Virus movement through plasmodesmata (PD): The movement protein of the virus is essential for the virus to pass through the plasmodesmata when passing from one cell to the other. The PD diameter is about ~1kDa is too small to allow passage of viruses. But viruses have the ability to enlarge the PD in certain cases by "PD gating" or formation of tubules. In spinach *Tobacco mosaic virus* (TMV) remains localized in the lesion, but if its capsid protein is exchanged with that of *Alfalfa mosaic virus* the virus moves. The TMV capsid protein does not enable movement through the PD. Treatment of tobacco nn, in which TMV moves systemically, with SA reduces TMV, apparently due to lower translocation through the PD. Transformation of tobacco with the salicylate hydroxylase gene, that prevents SA accumulation, causes spreading of TMV.
- Hypersenstive response (HR): The necrotic local lesion is one of the most notable resistance mechanims. TMV in tobacco NN is limited to the lesion, but occurs outside the necrotic area. When plants are placed at a temperature above 30°C, the virus moves to other parts of the plant. Before appearance of the necrosis electrolyte leakage is observed. Caspase like proteases involved in programmed cell death (PCD) in animals participate in the HR. Caspase inhibitors induce systemic movement of the virus. It was shown that a vacuolar protease (VPE) has caspase-like activity and may trigger HR.
- The N gene: The N gene was isolated from *Nicotiana glutinosa* by transposon tagging, and codes for a 131k Da protein. The N gene belongs to the TIR-NBS-LRR of the R genes. Transferring the N gene to tomato conferred resistance to them. The N protein identifies the C-terminal helicase domain of the replicase and starts a signal transduction cascade, where more proteins are produced that inhibit virus replication.
- Inhibitor of virus replication (IVR): A protein termed IVR was found to be associated with resistance. IVR protein was isolated, an antiserum against it was prepared, which served for isolating an IVR clone NC330. Transformation of susceptible tobacco plants with the IVR gene yielded plants with varying degrees of resistance. Some of the TMV-resistant plants were also resistant to *Botrytis cinerea.* R genes to viruses are known today to confer resistance also to fungi. A gene similar to the IVR gene in tobacco was subsequently also located in *Arabidopsis.*

## OP-27: A new pathotype of *Pepper mild mottle virus* (PMMV) overcomes the $L^4$ resistance genotype of pepper cultivars

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The biological, serological and molecular characteristics of a newly isolated $L^4$ resistance-breaking isolate of *Pepper mild mottle virus* (PMMV) were studied. This new pathotype of PMMV is serologically closely related to the Israeli pathotypes $P_{1,2}$ and $P_{1,2,3}$ of the virus, and has an identical host range. The mosaic symptoms caused by this new pathotype on pepper plants carrying alleles for resistance conferred by the L gene were more severe than the mild mosaic symptoms caused by other common strains of the virus. The amino acid sequence of the putative coat protein (CP) of the newly described pathotype has four and two amino acid differences when compared with the CP of the isolates $P_{1,2}$ and $P_{1,2,3}$ respectively. Based on its biological characteristics, the new isolate was designated pathotype $P_{1,2,3,4}$ of PMMV-Is. The three PMMV pathotypes could be distinguished from one another by restriction fragment length polymorphism of their CP genes.

## OP-28: Multiscale modelling of the emergence of virulent virus populations: towards new breeding strategies for building durable virus resistance

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Among the methods available to control plant virus diseases, using virus-resistant cultivars is certainly the only one combining both simplicity of use, low cost and low environmental impact. However, resistance genes are sometimes broken down by the emergence of adapted (*i.e.* virulent) individuals within virus populations. Accordingly, sources of resistance genes being limited and breeding for resistance being a long and costly process, the improvement of breeding strategies requires the use of new criteria to identify and manage durable resistance genes. One way to develop those criteria consists in ranking the various epidemiological, genetic and evolutionary factors involved in the emergence of virulent viruses according to their relative importance. In this modelling study, we investigate the relative importance of ten factors on resistance durability by means of two global variance-based sensitivity analysis techniques: Sobol and ANOVA. Sampling procedures use a quasi Monte Carlo sequence and a fractional factorial design. Specifically, we consider a case where the breakdown of a qualitative resistance gene requires the accumulation of mutations in $k$ nucleotide sites in the viral genome. At these $k$ nucleotide sites, each virus individual has the nucleotide required for virulence or not. Accordingly, there is $N = 2^k$ virus variants of interest for the study of resistance breakdown in the virus population. A model that simulates the demo-genetic dynamics of these $N$ variants at both within plant and between plants scales during an epidemic was developed. Within plant, selection, genetic drift, mutation and demographic stochasticity processes shaping the virus population are described by means of a system of $N$ differential stochastic equations. At the between plants scale (*i.e.* field), the epidemiological dynamics is described using an individual-based approach. The factors taken into account are: (1) the number of nucleotide sites involved in resistance breakdown, (2) the nature of the mutations involved (transition or transversion), (3) the mutation rate, (4) the transition to transversion ratio, (5) the virus carrying capacity of plants, (6) the fitness cost associated to the mutation involved in virulence, (7) the size of population bottlenecks (during the colonization of different organs within an infected plant or during plant-to-plant transmission by vectors), (8) the frequency of within plant drift events, (9) the contact rate between healthy and infectious plants and (10) the latent period (time necessary for an infected plant to become infectious). The first results suggest that the number of nucleotide sites involved in resistance breakdown is the most important factor explaining resistance durability followed by the fitness cost of mutations. Sensitivity analysis results are presented and their implications on breeding strategies discussed.

**Keynote presentation**

## OP-29: Epidemiology and integrated management of persistently aphid-transmitted legume and cereal viruses in West Asia and North Africa

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Cool-season food legumes (faba bean, lentil, chickpea) and cereals (barley and wheat) are the most important and widely cultivated crops in West Asia and North Africa (WANA), and are the main source of carbohydrates and protein for a large majority of the population. Luteoviruses pose a significant limitation to legume and cereal production worldwide. Surveys conducted in many countries in WANA during the last two decades indicated that the most important legume luteoviruses are: *Bean leafroll* (BLRV), *Beet western yellows* (BWYV) and *Soybean dwarf* (SbDV) *viruses*; and cereal luteoviruses are: *Barley yellow dwarf viruses* (BYDVs). The main symptoms produced by these persistently aphid-transmitted viruses are leaf yellowing, plant stunting, reddening, thickening of the leaves, and reduced flowering and pod-setting. Loss in yield is high, especially when infection occurs early. Monitoring of aphid flight activity in different regions indicated that *Rhopalosiphum padi* L., *R. maidis* Fitch., *Metopolophium dirhodum* Metoder. and *Sitobion avenae* Fabriciu, major pests of wheat and barely and known vectors of BYDV, were captured in all regions. Positive linear correlation was observed between BYDV incidence and the captured aphids that are able to transmit BYDV. The major aphid species in legume fields were *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch and *Aphis fabae* Scopoli. In addition, many wild species (annual or perennial) were found infected with luteoviruses and may play an important role in the spread of these viruses. In recent years, virus epidemics were reported in some countries of WANA, sometimes causing considerable yield reduction. Epidemic spread of these diseases was always associated with high aphid vector populations and activity. Although virus disease management can be achieved through the combined effect of several approaches, development of resistant genotypes is undoubtedly one of the most promising control components. The development of a sensitive, rapid, economical and simple technique to identify resistant genotypes is attractive to breeders, especially when a large number of genotypes need to be evaluated. Over the last decade barley and wheat genotypes resistant to BYDV, faba bean genotypes resistant to BLRV, and lentil genotypes resistant to BLRV and SbDV have been successfully identified. In addition, a relatively quick and simple plastic house technique was developed to identify resistant genotypes on the basis of relative virus movement and multiplication using Tissue blot immunoassay (TBIA). Moreover, progress was made in disease management of some of these viruses using a combination of management options. Many field experiments were conducted at different locations in the region to investigate the effects of a number of management components, such as planting date, plant density, seed dressing with Gaucho (Imidacloprid), and foliar spray with insecticide and mineral oil. Results showed that virus incidence was reduced and grain yield was increased when barley seeds were planted early compared to late planting, whereas virus incidence in faba bean fields in early planting was higher than that of late planting. Low plant density led to higher virus incidence than high plant density in both barley and faba bean fields. Seed treatment with Imidacloprid effectively reduced virus incidence in both faba bean and barley fields. Experience gathered over the last few decades clearly showed that no single method of virus disease control suffices to reduce yield losses in legume and cereal crops.

## OP-30: Development and evaluation of transgenic groundnut for resistance to *Tobacco streak virus* (TSV)

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Stem necrosis disease first recognized in the year 2000 caused by *Tobacco streak virus* (TSV; *Ilarvirus*) has wiped out almost all the groundnut harvest in 225,000 ha in Anantapur and Kurnool districts of Andhra Pradesh, India. Since then the virus has emerged as a major problem on groundnut and several other annual crops in Peninsular India. Due to the lack of TSV resistance in cultivated germplasm, a transgenic approach was undertaken to develop resistance against the virus in groundnut. The coat protein gene of TSV (*TSVcp*) cloned under the CaMV35S promoter in pCAMBIA2300 was introduced into the de-embryonated cotyledons of three popular groundnut varieties (JL 24, TMV 2 and ICGV 91114) by *Agrobacterium*-mediated transformation. Eighty percent of the resultant primary transgenic events ($T_0$) contained the *TSVcp* gene. Analysis of genomic DNA of 10 independent transgenic plants ($T_0$) demonstrated the integration of the *TSVcp* gene at one (in three events) to three (in seven events) loci within the genome. In Western-immuno assays using anti-TSV serum a polypeptide of ~50 kDa (presumed to be a dimer of *TSVcp*) was detected in 29% of the 92 primary transgenic events tested. Virus resistance assays with $T_1$ transgenic plants and non-transgenic controls (groundnut cv. JL 24) were done by mechanical sap inoculation at the three-leaf seedling stage (10 to 12 days after emergence). The inoculated leaves of the transgenic and non-transgenic controls showed necrotic lesions within 5 to 10 days post inoculation and they tested positive to TSV. However, three types of systemic reactions were observed in the transgenic events: (i) events with total lack of systemic symptoms and no virus accumulation; (ii) events that showed delayed systemic symptoms compared with the non-transgenic controls; and (iii) susceptible phenotype similar to that of non-transgenic controls within 15 to 20 days of inoculation. Through this procedure all the susceptible transgenic lines (including those showing delayed symptom expression) were eliminated from further resistance testing. These preliminary results suggest that the *TSVcp* mediates resistance against the systemic spread of TSV in certain events. The detailed phenotypic evaluation and molecular characterization of the resistant transgenic events and their advancement to further generations are in progress.

## OP-31: Transcomplementation and synergism in plants: implications for viral transgenes?

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In plants, viral synergisms occur when one virus enhances infection by a distinct or unrelated virus. Such synergisms may be unidirectional or mutualistic but in either case synergism implies that protein(s) from one virus can enhance infection by another. A mechanistically related phenomenon is transcomplementation, in which a viral protein, usually expressed from a transgene, enhances the infection of a virus from a distinct species. To gain an insight into the characteristics and limitations of these non-specific helper functions of individual viral genes and to assess their effects on the plant/pathogen relationship we compiled more than 150 published reports (dating back to 1945) of successful synergism, transcomplementation and viral gene exchange experiments. Results from these experiments were tabulated to highlight the phylogenetic relationship between the helper and dependent viruses and, where possible, to identify the protein responsible for the altered infection process. Analysis of the compiled data revealed that: (1) diverse viral life-cycle traits can be enhanced by synergism and transcomplementation. These include the expansion of host range, acquisition of mechanical transmission, enhanced specific infectivity, enhanced cell-to-cell and long-distance movement, elevated or novel vector transmission, elevated viral titre and enhanced seed transmission; (2) transcomplementation and synergism are mediated by many viral proteins, including coat proteins, replicases, movement proteins and proteins that disable host defences; (3) although more frequent between closely related viruses, transcomplementation and synergism can sometimes cross almost all known phylogenetic boundaries; (4) lastly, specific viral proteins can transcomplement multiple viruses and when this occurs transcomplementation can confer or enhance different life-cycle traits on each individual virus. Transcomplementation and synergism are of general interest as indicators of the interoperability of viral genes but these results can also be applied to risk assessment of transgenic crops expressing viral proteins. Most viral transgenes are inserted for the purpose of conferring viral resistance and are believed to operate via a gene silencing mechanism. Silenced transgenes can however transcomplement non-target viruses. Thus transcomplementation may occur in resistant plants and may lead either to enhanced infection by the normal range of viral pathogens or it may render the transgenic crop susceptible to viruses against which it would normally be resistant. This study contributes to the characterisation and identification of such potential hazards and can be used to identify data gaps and other limitations in predicting the likelihood of transgene-mediated transcomplementation.

**Invited presentation**

## OP-32: Management of the island sugarcane planthopper, *Eumetopina flavipes* (Delphacidae), vector of Ramu Stunt disease of sugarcane in Papua New Guinea

**Kylie L. Anderson**[1], Mohamed Sallam[2], Robert C. Magarey[3] and Bradley C. Congdon[1]

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The island sugarcane planthopper, *Eumetopina flavipes* is the only known vector of Ramu stunt disease of sugarcane (*Saccharum officinarum*), which has been responsible for production losses of up to 60% at Ramu Sugar Ltd in Papua New Guinea (PNG). Ramu stunt disease and *E. flavipes* are widespread on sugarcane throughout PNG, but disease free populations of *E. flavipes* occur throughout the Torres Strait Islands (a well recognised Australian quarantine front-line) and on the north-eastern tip of mainland Australia. *E. flavipes* constitutes an economic threat to both the growing PNG and vital Australian commercial sugarcane industries given its vector status and proximity to major cane-growing areas, and is listed as a high priority on state and federal Australian quarantine target lists. Factors which may influence dispersal of *E. flavipes* throughout its range have been identified as distance from PNG, host distribution and abundance, wind assisted dispersal, and historical versus contemporary people movement. Each of these factors provides a distinct, predicted pattern of *E. flavipes* distribution and abundance which may be compared to real *E. flavipes* distribution and abundance data, allowing a relative evaluation of their fit. The proportion of sugarcane plants infested by *E. flavipes* appears to be influenced by distance from PNG, but this relationship is driven by several highly infested islands close to the PNG coast. If these islands are removed, the relationship is not significant. There is no significant effect of distance from PNG on *E. flavipes* abundance. On infested islands, there is no significant effect of the proportion of infested sugarcane plants, or the availability of sugarcane plants on the abundance of *E. flavipes*. Therefore, distance from PNG and host abundance appears to have no little or no effect on the distribution and abundance of *E. flavipes*. However, the fact that seven of the twenty islands contain abundant host but are devoid of *E. flavipes* remains unexplained. Preliminary analysis indicates no significant relationship between wind direction and *E. flavipes* abundance and distribution. Therefore, dispersal and associated colonisation does not appear to be strongly influenced by natural processes as described above. Of predicted *E. flavipes* distribution and abundance under historical or contemporary people movement, the former provides the best fit when compared to current *E. flavipes* distribution and abundance data. These results and other life history characteristics may be synthesised into a descriptive ecological model which may allow the prediction of incursion points and hot-spots, not only for Ramu stunt disease and *E. flavipes*, but for organisms which display similar ecology. The model provides a tool which may assist in the development of surveillance and monitoring programs, integrated management plans and appropriate control or eradication response.

## OP-33: The challenges and potential options for controlling virus diseases of legumes and tuber crops in West Africa

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Tuber crops, cassava (*Manihot esculenta*) and yam (*Dioscorea* sp.), and grain legume crops, cowpea (*Vigna unguiculata*) and soybean (*Glycine max*) are amongst the major crops cultivated in a combined area of 18.74 million ha in West Africa (WA). Productivity of these crops in WA is often low and unpredictable due to several biotic, abiotic and socio-economic factors. Among these are plant virus diseases that pose a significant threat to crop productivity, quality and impede safe movement of germplasm. Cassava mosaic geminiviruses (CMGs; *Begomovirus*) on cassava; *Cucumber mosaic virus* (CMV; *Cucumovirus*), *Dioscorea alata bacilliform virus* (*Badnavirus*) and *Yam mosaic virus* (YMV; *Potyvirus*) on yams; *Blackeye cowpea mosaic virus* (*Potyvirus*), CMV, *Cowpea mosaic virus* (*Comovirus*), *Cowpea mottle virus* (*Carmovirus*), *Cowpea aphid-borne mosaic virus* (*Potyvirus*) and *Southern bean mosaic virus* (*Sobemovirus*) in cowpea are regarded as the most economically important viruses in WA. Although more than 10 viruses have been reported to infect soybean in WA, information on their economic significance is limited. The viruses infecting these four crops have been extensively studied over past several decades to characterize the viruses and develop diagnostic tools. However, research focus on virus biodiversity, disease epidemiology and disease control options, particularly through development of host plant resistance, have been limited to a few viruses such as CMGs of cassava and YMV of yams. Significant advances have been made in development of sensitive virus detection tools, development of virus-free planting material through tissue culture procedures and use of phytosanitary procedures for safe movement of germplasm, which have contributed to the distribution of pathogen-free propagation materials to farmers. Despite availability of a range of solutions to manage viral diseases, they remain as major problem at field level mainly due to poor adoption of available technologies. In several cases effectiveness of the management options were limited by gaps in knowledge on nature and prevalence of virus types/strains, the role and seasonal dynamics of its vectors and their epidemiology. For instance, information on viruses infecting yams, cowpea and soybean are inadequate on the prevalence, distribution and spread and the losses they cause. There are likely to be many undiscovered viruses infecting these crops. Infections with multiple virus species/strains are relatively common in these four crops in different regions in WA. This situation complicates and even compromises the resistance breeding programs and quarantine. In addition, lack of breaks in over-seasoning, large diversity of crops and varieties grown, the wide host range of viruses and their vectors, inadequate capacity for technology development/adoption and phytosanitary certification are some of the major challenges for virus disease control in WA. Available options for development of a range of management tactics will be discussed.

## OP-34: Centennial of research on groundnut rosette disease: what is known and what still needs to be known to achieve effective control of this menace in Sub-Saharan Africa

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Several economically important plant virus diseases involve synergistic interactions between causative viruses, wherein the presence of one virus facilitates the other virus deficits in some molecular function for survival and spread. Groundnut rosette disease (GRD), first reported in 1907 from Tanganyika (presently Tanzania), is the most fascinating example of such an interaction, wherein three agents are intricately dependent on each other for survival and spread. GRD has been recognized in the all groundnut growing countries on the African continent, including its offshore islands such as Madagascar, but not anywhere outside Africa. GRD is responsible for annual groundnut loss of worth US$ 150 million. The disease occurs in two predominant symptom forms - chorlotic rosette and green rosette, infection of which at early growth stage results in up to 100% yield loss. GRD is caused by a complex of three agents: *Groundnut rosette assistor virus* (GRAV; Family, *Luteoviridae*), *Groundnut rosette virus* (GRV; Genus, *Umbravirus*) and its Satellite-RNA (satRNA, Sub-Group 2 Satellite RNA). GRD symptoms are associated with the presence of the GRV-satRNA complex, in which variants of the satRNA are responsible for different symptom types, such as chlorotic rosette, green rosette and mottle rosette. GRAV replicates autonomously in plants and is transmitted by an aphid, *Aphis craccivora* Koch (Homoptera: Aphididae). GRAV alone causes no obvious symptoms, but it was shown to cause substantial yield reduction in susceptible genotypes. The satRNA depends entirely on GRV for its replication, and GRV must be associated with its satRNA for its packaging in the GRAV coat protein and subsequent transmission by the aphid vector. Through the ability to utilize the coat protein of GRAV, GRV-satRNA gains epidemiologically by acquiring a persistent relationship with the aphid vector for survival and spread. Resistance sources to GRD have been found in cultivated as well as wild *Arachis* germplasm. Breeding for host plant resistance at ICRISAT have contributed to the development of several groundnut varieties with acceptable levels of field resistance. However, all the resistant genotypes identified apparently contain the same genes conferring resistance to GRV & satRNA, and several of them are long duration types. There is a need to broaden the genetic base of GRD resistance to avert any breakdown of resistance under severe disease pressure, and breed short-duration GRD resistance varieties preferred by the farmers in SSA. This paper illustrates the successful international collaborative research efforts since the first report of GRD 100 years ago in unraveling the etiology, molecular mechanism of interaction between the agents and its invertebrate vector, leading to the development of control strategies. The paper will further emphasize the need for diverse approaches required to understand the critical information pertaining to the off-season survival of the disease agents and aphid vector and the need for new GRD resistant cultivars, if effective control of this major plant virus disease of groundnut in SSA is to be achieved.

## OP-35: Screening groundnut varieties for resistance to the groundnut rosette disease

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Groundnut rosette disease (GRD) is an important disease constraint to groundnut production in sub-Saharan Africa with epidemics often resulting in 100% yield losses. Following initial reports in Tanzania in 1907, GRD has to date been confirmed in several African countries including DR Congo, Gambia, Kenya, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Uganda, Zambia and its off shore islands. No conclusive evidence has been presented of occurrence of GRD outside Africa. Various strategies have been utilized to manage the GRD problem including use of pesticide applications to reduce aphid vector populations; varying cropping practices to delay on-set and spread of the disease and breeding for virus/vector resistance in the host plant. The most practical approach for small-farm holders in SSA still remains use of host plant resistance as a primary component of an integrated disease management strategy. Although various resistant varieties have been bred and deployed by ICRISAT, there is need to broaden and strengthen available resistance strategies by identifying further sources of resistance. In addition, for resource poor farmers, it would be important to identify varieties with resistance to multiple diseases. Experiments were conducted during the 2004/05, 2005/06, 2006/07 cropping seasons at Chitedze Agricultural Research Station, a GRD and Early leaf spot (*Cercospora arachidicola*) hotspot location in Lilongwe, Malawi. When complemented with artificial inoculations through the infector row technique screen, Chitedze provides an excellent screening environment. Malawi is characterized by a mono-modal rainfall pattern. The purpose of the study was to evaluate response of 143 accessions to GRD. Six accessions- four resistant (ICGV-SM 90704; ICGV-SM 99568; RG1; ICG 12991) and two susceptible (JL24 and Malimba)- were used as control plants. Data was collected on GRD incidence (%), pod weight (kg/ha), haulm weight (kg/ha), kernel yield (kg/ha), shelling percentage (%), 100 seed mass (g), and Early leaf spot (ELS) at 100 days. Study results revealed significant differences (P>0.001) in disease levels among varieties tested. In all varieties in which disease was recorded, similar symptom expression was observed involving leaf chlorosis, distorted, and folded leaflets together with stunting. GRD symptoms were observed on 130 of the 143 accessions tested where disease incidence varied from 0% to 100% in individual plots. Out of the 143 accessions, 94 were scored as susceptible (50%-100% incidence); 16 as moderately susceptible (31-50%); 5 moderately resistant (11-30%) and 28 as resistant (<10%). A significant and negative correlation (P<0.001) was observed between kernel yield (kg/ha), 100 seed mass (g) and GRD incidence implying that rosette incidence significantly decreased kernel yield. In addition, the results also suggest that the smaller seeded Spanish and Valencia types showed higher susceptibility to GRD than Virginia types. Two Virginia bunch accessions, ICGV-SM 91707 and RG-1 (VB) were classified as resistant to both GRD and ELS. Implications of the results for breeding for multiple disease resistances to foliar diseases in groundnuts are discussed.

## OP-36: Good agricultural practices (GAPs) for successful management of *Papaya ringspot virus* (P) in papaya - a case study of IARI regional station, Pune, India

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*Papaya ringspot virus* (P) (PRSV) infection is a serious threat to papaya (*Carica papaya* L.) cultivation throughout the world. In an estimate, 49.05% yield losses due to PRSV were recorded when plants were infected between flowering and fruit set and 38.38% loses when plants were infected after fruit set when compared with plants infected after fruit development stage. Unfortunately, there is no prophylactic or therapeutic control of PRSV infection. The only solution to minimize yield losses is to adopt an integrated approach involving various factors of cultivation. Based on the trials at IARI, Regional Station (RS), Pune and the experience gained from the farmers' fields, a package of Good Agricultural Practices (GAPs) was developed to minimize losses due to PRSV infection. GAPs are aimed at delaying PRSV infection. They start with the selection of the cultivars that produce better yield in PRSV infection. Presently, the cultivar Red Lady is performing better than other cultivars. PRSV infection at seedling stage is common in many nurseries. PRSV infected seedlings usually produce poor yield of inferior quality fruits, therefore, use of virus-free seedlings is the base foundation for GAPs. PRSV is mainly transmitted through aphid-vectors, therefore, GAPs are oriented towards avoiding infection sources and aphid-vectors. Infection sources can be avoided by planting a new papaya plantation away from other host plants like cucurbits and infected papaya plantation. In real farming condition, it is difficult to maintain isolation distance. In a trial at IARI RS Pune, papaya production increased marginally in the vicinity by rouging infected plants early. Aphid-vectors can be avoided by growing a border crop around papaya plantation, and adjusting the season of transplanting. The border crop should be at least eight feet tall, last for two years, and should be 3-4 feet high at the time of papaya transplantation. Border crop reduces entry of aphid-vector population (thereby, delaying PRSV infection). It creates a micro-environment congenial for papaya cultivation, and works as the wind break, for fruit plants are prone to uprooting by fast wind. The border crop of banana reduced the aphid population (weekly total/trap) from 84.42, outside to 44.04 inside the border crop. Aphid-vectors exhibit a seasonal trend in their population. By transplanting papaya during the lean period (spring season), PRSV infection can be delayed till monsoon, by that time plants have crossed the fruit bearing stage. Incidence of PRSV till fruit set was minimum on plants transplanted in early spring (18.25%), followed by plants transplanted on mid (22.91%) and late (33.33%) spring. Regular application of insecticides failed to affect PRSV incidence significantly in trials at IARI RS Pune. Cultivation of PRSV resistant transgenic cultivars (SunUp and UV Rainbow) could overcome the problem only in the limited geographical location (Hawaii, USA). Use of transgenic cultivars as a parent in PRSV resistant breeding programme is raising hope for development of PRSV resistant cultivars, but it will take some time. Therefore, use of recommended GAPs for PRSV management can be the best bet for successful cultivation of papaya in spite of PRSV infestation.

# Session IX: Characterization and Diagnosis of Viruses & Vectors

### Invited Presentation

## OP-37: The identity of the leafhopper vectors in the genus *Orosius* Distant (Hemiptera: Cicadellidae)

**Murray J. Fletcher**[1] and Andrew Mitchell[2]

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The leafhopper genus *Orosius* Distant contains some of the best known vectors of plant phytoplasma and virus diseases, including phytoplasmas such as tomato big bud, lucerne witches broom, legume little leaf and potato purple top wilt as well as mastreviruses such as *Tobacco yellow dwarf virus* and *Chickpea chlorotic dwarf virus*. Unfortunately, misnomers and misidentifications have led to some confusion about which species occur in which regions of the world. Most confusion applies to the use of the name *Orosius orientalis* (Matsumura), described originally from Japan. Recent publications using this name from the Middle East are incorrect, the species occurring in this region being *O. albicinctus* Distant, which is distributed from the Middle East, North Africa to India. *O. orientalis* does not occur in this region. A proposed synonymy between *O. orientalis* and the well known Australian vector species *Orosius argentatus* (Evans) was met with caution by authors because no comparison had been made with Japanese material. This comparison, based on examination of male genitalia from several localities in Japan, has now been done and the synonymy between *O. argentatus* and *O. orientalis* is confirmed, *O. orientalis* having priority. *O. orientalis* is distributed in the eastern oriental and Australian regions but has not been recorded west of Java in Indonesia. We have been developing diagnostic protocols based on sequencing the COI gene region to confirm the identities of the known species, *O. orientalis, O. albicinctus, O. canberrensis* (Evans), *O. lotophagorum* (Kirkaldy) and three new species in Australia as well as to provide a method for accurately identifying adult females and nymphs. The status of *O. argentatus novaebrittaniae* Ghauri and *O. lotophagorum ryukyensis* (Ishihara) has yet to be determined. Other species yet to be bar-coded are *O. aegypticus* Ghauri and *O. cellulosa* (Lindberg).

## OP-38: Control of vectors and insecticide resistance: Implications for Disease control

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Effective management of insect and mite vectors of plant pathogens is of crucial importance to minimizing vector-borne diseases in crops. Insecticides play an important role in managing vector populations by reducing the number of individuals that can acquire and transmit a virus, thereby potentially lowering disease incidence. Certain insecticides also play a role in protecting crop plants by virtue of anti-feedant properties that interfere with virus transmission. Maintaining effectiveness of insecticides is a principal goal of pest management and more specifically resistance management. Some of the most notorious vector species are also highly prone to development of resistance to insecticides, foremost among these being *Bemisia tabaci* and *Myzus persicae*. Insecticides become less effective against vectors because of resistance mechanisms that metabolize them to non-toxic products or through alteration of enzymes making them insensitive to act. Such mechanisms may be similar across many vector taxa and species but can be potentially unique and complex for each species. As such, resistance management in each species should start with detection and surveillance for resistance by monitoring efforts. Towards that end, monitoring and detection for resistance to neonicotinoids was established in *Bemisia tabaci* populations from various geographic regions. Neonicotinoid insecticides belong to a novel class of insecticide chemistry and have been critical in whitefly and aphid management for the last decade worldwide. The impact of this new chemistry, which includes imidacloprid, thiamethoxam and acetamiprid, against whiteflies and aphids has been significant and economically beneficial to the growers in production agriculture and horticulture. Neonicotinoids act upon the nicotinic acetylcholine receptor of insects, a target that is not utilized by pyrethroids, carbamates and organophosphates. Therefore, populations of whiteflies and aphids showing high levels of resistance to conventional insecticides have proven to be highly susceptible to the neonicotinoids. However, with extensive and successful usage of these insecticides, there is potential risk for resistance. The issue of resistance to neonicotinoids was addressed by monitoring for susceptibility of whiteflies to determine resistance to each compound and cross-resistance between neonicotinoids. Results from this study showed differences in resistance and cross-resistance patterns to neonicotinoids in multiple strains of whiteflies. Although differences at the target site and/or metabolic pathways may influence the variability in cross-resistance patterns among whitefly populations, comparison of whitefly responses from various geographic regions to the four neonicotinoids indicates the importance of ecological and operational factors on development of cross-resistance to the neonicotinoids. Assessing specific instances of resistance in a particular whitefly population is important to allow control strategies to be adjusted to specific needs in a geographic area.

## OP-39: Diagnosis, epidemiology and management of *Watermelon bud necrosis virus* infecting watermelon

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Epidemic of bud necrosis, caused by thrips-transmitted *Watermelon bud necrosis virus* (WBNV), were monitored in watermelon (*Citrullus lannatus*) cultivars Madhu Bala, NS271 and Arka Manik, during 2002 to 2007 at IIHR Bangalore. Final incidence of bud necrosis and area under the disease progress curve values for Madhu Bala were similar to those for NS 271 but lower than for Arka Manik. AUDPC values were lower in Madhu Bala and NS 271 than A, Manik, rate of disease progress was show in Madhu bala and high in A. Manik where as it is intermediate in NS 271. Temporal analysis of epidemic and disease progress curves was compared with monomolecular, logistic and Gompertz models were evaluated for goodness of fit. Of all the models, Gompertz model described well the epidemics ($R^2$ 0.87) between the cultivars. Management practices evaluated were border cropping, planting date, and insecticide treatment and light reflective mulch. The effect of planting date was consistent in that the late planting resulted in higher incidence of WBNV and low yield. In all years' intensive early insecticide treatment combined with reflective mulch reduced thrips population, WBNV and increasing marketable yield compared with control. Although none was sufficiently effective alone, integrating all the practices were found significant in reduction of disease loses.

## OP-40: Transmission and epidemiology of *Papaya ringspot virus-w* (PRSV-W) infecting pumpkin

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*Papaya ringspot virus*-w (PRSV-W) infects a number of cucurbitaceous vegetables. PRSV-W is vectored by many transient aphids, chiefly *Aphis gossypii*, *A. craccivora* and *Myzus persicae* in a non-persistent manner. In India, systematic study on the aphid species involved in the spread of PRSV-W, transmission efficiency, cross-transmission to different host plants, marketable yield loss and effect on seed yield and quality is lacking. Studies were carried out from August to November 2005 (trial-1) and 2006 (trial-2) at the Indian Institute of Horticultural Research, Bangalore on the above aspects. The number of alate aphids in nature were monitored every 24 hr using yellow funnel water traps. PRSV-W incidence on pumpkin was recorded by tagging newly infected plants at weekly interval. Further, aphid transmission from pumpkin to pumpkin and other cucurbitaceous vegetables was carried out using *Aphis gossypii*, *A. craccivora* and *Myzus persicae.* PRSV-W infection was further confirmed by DAC-ELISA. Fruits from the tagged infected plants were harvested individually to understand time of infection influencing fruit yield, seed yield and quality. Sampling for migrant alate aphids indicated that *Aphis gossypii* was observed to be the dominant species in yellow funnel traps (>70 % in both the trials). Significant correlation was observed between numbers of *A. gossypii* trapped 4 weeks prior and new PRSV-W infection. Early infection (6 weeks after planting) significantly reduced pumpkin fruit weight by >60%. Further, depending upon the time of infection, number of fruits per plant, fruit diameter and number of seeds per fruit reduced significantly. Transmission efficiency varied among aphid species while transmitting PRSV-W to different cucurbits. Cross transmission studies indicated aphids successfully acquire and transmit PRSV-W to muskmelon (*Cucumis melo*), watermelon (*Citrullus lanatus*), ridge gourd (*Luffa acutangula*), ash gourd (*Benincasa hispida*) and cucumber (*Cucumis sativus*) and bottle gourd (*Lagenaria siceraria*) with significantly (30 to 100%) high efficiency. However, the efficiency was less (10%) while transmitting to bitter gourd (*Momardia charantia*).

**Keynote presentation**

## OP-41: Epidemiology of leafhopper-borne *Maize yellow stripe virus* in Egypt

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*Maize yellow stripe tenuivirus* (*MYSV*), which was recorded in 1983 for the first time on Egyptian maize fields, is discussed for its ecology and epidemiology throughout 25 years. The virus has been found on Single-infected plants in summer season (May-September) as major growing season. Higher infection rate (≤ 20%) were observed in Nily season (August-November). *MYSV* infection kept in low rate (5-7%) as single infected plants for 8 years. Maize fields, grown in summer season of 1991, were suffered from high infection rate (50%-75%) of yellowing, stunting, and leaf stripping in Middle Egypt (Giza, Fayium, Beni Suef and Minia). Too many fields had been removed by the farmers because of infection effects. It was concluded that *MYSV* was the causal of that severe epidemic. *MYSV*-infection rate were then getting higher (≥ 7%) in maize fields of summer season throughout 9 years. Severe infection (65%-85%) has been recorded in 2000 on summer maize fields of Beni Suef, Sahag and Quena. Maize plants were stunted, leaves were stripped, apical stems were curled and kernels were not produced or produced with non-marketable value. Biological and serological analysis proved that the causal was *MYSV*. This virus is transmitted by adult and nymphs of *Cicadulina chinai* leafhopper in a persistent propagative manner. Peaks of *C. chinai* population fluctuations were recorded in October and November (during Nili season of maize plantations). Virus-vector relationship has been extensively studied. Recently, in 2004 and 2005 growing seasons, disease severity and percentage of MYSV incidence in ten governorates were recorded using visual examination of MYSV symptoms, serodiagnosis using DACELISA, and sometimes molecular tools (IC/RT-PCR and hybridization) to estimate ecological and epidemiological factors of MYSV infection. Generally, MYSV occurrence was higher in Middle Egypt than in Lower Egypt. Also, it was higher in Nily than in summer plantations (≥ 15%). However, MYSV incidence in the summer season of 2004 was higher than in Nily season of the same year. Epidemiology and insect-dependent transmission of this virus by *Cicadulina chinai* is discussed.

## OP-42: Emergence of new phytoplasma and virus diseases

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Our earlier tests have demonstrated that highly specific leafhopper vectors lose their diet restriction after feeding on phytoplasma diseased non-host plants. The altered feeding permitted vectors to survive on previously unsuitable and unacceptable plants. Vector access to new host plants might thus provide a mechanism for the emergence of new vector-borne phytoplasma or virus diseases. A second mechanism for the creation of novel virus or phytoplasma diseases could be created by several species of plant-parasitic dodder (*Cuscuta* spp.). Dodders can graft themselves to various new hosts, not infected naturally by leafhopper or plant hopper vectors. The two mechanisms, separately or in combination, could lead to the emergence of unexpected new plant diseases as well as new vectors. To prevent or diminish the possible emergence of such novel phytoplasma and virus diseases of cultivated crops, manual destruction of dodder spp. and rouging of phytoplasma diseased plants is recommended.

## OP-43: Comparison of high throughput PCR and tissue blot immunoassay for large-scale virus field surveys

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A virus survey of pea and faba bean crops provided the opportunity to efficiently process large numbers of field samples and compare the diagnostic results using a rapid, high throughput reverse-transcriptase PCR (RT-PCR) method with those obtained using tissue blot immunoassay (TBIA). Random plant samples were tested for *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Pea seedborne mosaic virus* (PSbMV), *Bean leafroll virus* (BLRV) and *Beet western yellows virus* (BWYV) by TBIA. All samples were also tested by RT-PCR for PSbMV and BLRV, which were previously identified in diagnostic samples from the region. Hundred random tendril or petiole samples were taken from each of 21 pea crops and 3 faba bean crops in southern NSW, in October 2006 and bundled into 10 groups of 10 with Parafilm. Data was entered onto a database on a PDA. Tissue bundles were blotted on nitrocellulose membranes and TBIA was conducted using a standard protocol with virus-specific antibodies (DMSZ, Germany). RNA was extracted from each tissue bundle using the modified MacKenzie method, by centrifugation in 96 well silica Unifilter® microplates. The RT-PCR reactions were set up with the Corbett CAS 1200 robot in 25µl volumes containing specific primers for BLRV or PSbMV. PCR products were separated on 1.5% agarose using the ElectroFast® high throughput gel tanks (ABgene) and visualised under UV with SYBR® safe DNA gel stain (Invitrogen). The three sampled faba bean crops had a high incidence of BLRV (28-42% infected plants), while one crop also had 6% BWYV. High levels of PSbMV (22-77% infected plants) were found in 12 of the 21 pea crops. BLRV and BWYV occurred in 12 and 15 pea crops respectively and the percentage of infected plants ranged from 1-63%. Other viruses were absent or insignificant in both faba bean and pea crops. The bundling of plants into groups of 10 was an efficient way of using the same plant tissue for blotting and RNA extraction. The whole PCR process from sample preparation to results for 2400 samples was completed in 14 hours. The rate-limiting steps in the diagnostic processes were the physical grinding of the samples for RNA extraction and the blotting for TBIA, while other costs were comparable. Statistical tests showed that both methods reliably detected the presence or absence of virus in the crops. The TBIA results were assumed ‘true’ and the sensitivity and specificity of the PCR tests were 96% and 100%, respectively, for PSbMV and 96% and 95%, respectively, for BLRV. The ability to use both PCR and TBIA on a sample set will greatly enhance the flexibility in approaching surveys. The use of high throughput RT-PCR was effective for the large-scale diagnosis of viruses in pulse crops and will be particularly applicable when highly specific molecular diagnostic tests are required, such as during response to an incursion of an exotic pathogen.

## OP-44: Development of a diagnostic multiplex IC-RT-PCR system for the differentiation of *Potato virus Y* strains

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*Potato virus Y* (PVY) is one of the most common and destructive viruses found in potato. According to symptoms induced in indicator plants virus isolates have historically been divided into three main strain groups: $PVY^{O}$, $PVY^{N}$ and $PVY^{C}$. In the last two decades new PVY variants appeared, including two groups of recombinant strains: $PVY^{N}$-W and $PVY^{NTN}$, and NA-$PVY^{N/NTN}$. Available biological and molecular data on PVY suggest that classification of PVY strains has to be revised. Some drawbacks have been found with recently published primers used in RT-PCR based differentiation of PVY strains as some defined isolates could not be identified correctly. To investigate epidemiological aspects of appearance and distribution of different variants, to understand their evolution and to find new sources of resistance, reliable and rapid diagnostic systems are necessary. A test system, based on a complex of biological, serological and IC-RT-PCR techniques was proposed. New primers using both recently available sequences and newly generated complete sequences of PVY strains were developed. Strains $PVY^{N}$, $PVY^{O}$, $PVY^{C}$ and NA-PVY $^{N/NTN}$ can be discriminated using their sequence differences, the recombinant variants $PVY^{N}$W and $PVY^{NTN}$ can be differentiated from their parents solely applying different positions of the recombination points. Diagnostic primer pairs reflected the known situation of recombinant variants. All designed primers proved to be highly specific and did not detect unrelated variants in the case of single as well as in artificial and natural mixed infection. The reliability of these newly developed primers and test procedures were successfully demonstrated on nearly 200 biologically and serologically characterised PVY isolates from around the world. To simplify the diagnostics of PVY and to reduce processing costs, multiplexing analysis using primers developed was introduced. Reliable results were obtained for quadruplexing of isolates N-, O-, C- and NA-N/NTN variants. It was not possible to include $PVY^{N}$W and recombinant $PVY^{NTN}$ variants in a combined multiplex test format because of the size of the necessary PCR products. To differentiate these isolates a separate duplex PCR had to be set up. The advantage of the presented system consists mainly in the fact that any mixed infection can be clearly defined.

## OP-45: Occurrence and PCR detection of cassava mosaic geminiviruses on *Jatropha curcus* in Tamil Nadu

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*Jatropha curcus* is a drought-resistant perennial crop which produces seeds with an oil content of 37%. During 2007 Kharif at Erode district (Tamil Nadu) we observed the incidence of mosaic disease in the naturally grown jatropha plants in the cassava fields. The infected leaves exhibited crinkling, curling, chlorotic lesions near the veins and stunting which was similar to the symptoms of geminivirus infection, and hence the total DNA from the infected plant was extracted and the occurrence of the geminivirus was confirmed with the help of Deng primer (degenerate primer) in PCR assays. Consistent amplification of DNA-A fragment was obtained (c. 500 bp fragments) from symptomatic plants. Then the infected plants were subjected to multiplex PCR analysis using primers specific to *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV). This resulted in specific amplification of 904 bp and 599 bp precuts of ICMV and SLCMV, respectively, from the symptomatic jatropa plants, but not from healthy looking plants. The PCR analysis revealed the association of cassava mosaic geminivirus in jatropa plants showing mosaic disease.

## OP-46: Genetic diversity of Banana bunchy top virus in India

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*Banana bunchy top virus* (BBTV) is one of the most important diseases of banana in the world causing enormous economic loss to bananas and plantain. It has been reported from all major banana growing countries. In India, BBTV has almost wiped-out the famous hill banana viz., Virupakshi (AAB) grown in Lower Pulney hills and Sirumalai hills of Tamil Nadu. This disease could not be easily eradicated in the region owing to its perennial nature of cultivation, presence of large viral inoculum and vector throughout the year. Based on the previous diversity studies, two groups have been identified viz., the isolates from Philippines, Vietnam and Taiwan belongs to Asian group and the south pacific group with the isolates from Australia, Fiji, Burundi and India. In this study, 5 BBTV isolates were collected from north-eastern hill states of India (Bunchy top symptomatic wild banana clones naturally occurring in Arunachal Pradesh, Meghalaya, cultivated clones from Nagaland, Assam) and Kodaikanal hills (BBTV infected hill banana) of Tamil Nadu, India and the BBTV coat protein (CP) gene from these isolates were amplified and cloned into pGEM-T easy vector. The CP genes were sequenced in both directions and the sequences were aligned using CLUSTALX software. The published sequence of BBTV CP from Bihar (India), Uttar Pradesh (India), Australia, Taiwan, Philippines, Vietnam and Fiji were used as representative sequences in this analysis. The comparative analysis of the Indian isolates with Asian isolates placed the Indian group in to a different cluster, indicating Indian isolates does not belong to Asian group. Cluster analysis of Indian isolates with South Pacific isolates, placed Meghalaya isolate into a different cluster. The highest percentage divergence of 6.5 was observed with Meghalaya and Australia. This indicated that BBTV isolate infecting wild banana though belonged to South Pacific group differed considerably with a mean divergence percentage of 3.58. The comparative analysis of all the three groups' viz., Asian, Indian and South Pacific isolates grouped into two clusters viz., Asia and South Pacific and Indian isolates with South Pacific. This analysis further confirmed that Indian isolates belong to South Pacific isolates. This study indicated the evolutionary relationship of Indian BBTV isolates with isolates from different geographical locations. High level of conservation has been observed in the BBTV coat protein amino acid sequences. This level of conservation indicates a promise for the successful application of PDR strategy in transgenics to combat the bunchy top disease in banana.

## OP-47: Molecular characterization of viral double-stranded RNA and plasmid-like DNA from the plant pathogenic fungus *Rhizoctonia solani*

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Extra-chromosomal genetic elements containing small molecular weight DNA (plasmids) and viral-like double-stranded (ds) RNA are widespread in plant pathogenic fungi. Weak pathogenic isolates of *Rhizoctonia solani*, a soil-borne fungus, showing abnormally slow growth were found to contain extra-chromosomal genetic elements while pathogenic isolates showing normal growth showed no detectable presence of plasmid DNA/dsRNA suggesting that extra-chromosomal genetic element might be a factor in the pathogenicity of the fungus. Our studies suggests that TE2-4, an isolate from AG 2-BI contain small plasmid DNA molecules in association with 3.3 kb viral-dsRNA. *R. solani* isolate TE2-4 belonging to AG-2BI were characterized in the present study. The genetic relationship of a 3.3 kb dsRNA to a 1.1 kb and 0.9 kb DNA were studied by nucleic acid hybridization. Plasmid DNA from the fungus was directly cloned into the pCAP$^S$ vector by generating random Polymerase Chain Reaction (PCR) blunt end products using Tgo DNA polymerase. A psoralin biotin labeled probe was developed using the 1.1 kb DNA plasmid in order to determine the genetic relationship of the dsRNA to the plasmid DNA. Nucleic acid hybridization showed no genetic relationship between the 3.3 kb dsRNA and the 1.1 kb plasmid DNA. Furthermore, nucleic acid hybridization results showed a correlation between the 1.1 kb and 0.9 kb fragments. This suggests both the 1.1 kb and 0.9 kb plasmids have similar sequences and may have either derived from the same source or has the ability to self-replicate making more copies. Sequencing analysis revealed the vector to contain a 730 bp insert. The partial DNA sequence will be used to construct a full-length DNA clone to further analyze and determine the role of plasmid DNA in disease attenuation.

## OP-48: Legume yellow mosaic viruses, genetically isolated begomoviruses: diversity of legume-infecting begomoviruses in Pakistan

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Four species of begomoviruses (whitefly-transmitted geminiviruses) (*Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* and *Horsegram yellow mosaic virus*) are responsible for yellow mosaic diseases of legumes across southern Asia. They cause losses to a number of important pulse crops which are a major source of dietary protein. These viruses are limited to plants of the family *Fabaceae* and efforts to reduce losses are hampered by limited availability of conventional, host-plant resistance sources and/or lack of durability of the resistance that has been identified. These viruses interact with each other within the legumes, in the form of classical recombination and component exchange (pseudo-recombination), is well documented. However, in contrast to the majority of the other begomoviruses, there is little, if any, evidence for interaction with non-legume infecting viruses. This is indicative of genetic isolation; the viruses in legumes evolving independently. This isolation also holds the promise of development of durable resistance. An ongoing project to investigate the diversity of legume yellow mosaic viruses in Pakistan has only identified MYMIV, despite an earlier report of the presence of MYMV in the country. The diversity of this virus is very low across a number of leguminous hosts. Additionally we have for the first time identified a non-legume begomovirus, *Tomato leaf curl Pakistan virus*, in soybean infected with, and showing symptoms of, MYMIV. This indicates a possible breakdown in the genetic isolation of the legume-infecting begomoviruses. The significance of these findings will be discussed.

# Session X: Molecular Epidemiology and Ecology

### Keynote presentation

## OP-49: The molecular epidemiology of cucurbit viruses

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Understanding the key factors of virus epidemics is an important step towards the development of sound control methods. The recent development of simple, rapid and relatively cheap molecular methods has greatly contributed to a better characterization of virus isolates occurring in field samples. These techniques associated to classical serological or biological methods presently offer the opportunity to reassess different steps leading to plant virus spread. This is particularly important for determining the origin of virus inoculum at the field level (reservoirs) or at a regional scale (emerging viruses). It also allows following the dissemination of new virus strains either recently introduced or recently emerged within local populations. Examples where molecular techniques have contributed to a better understanding of virus epidemiology will be provided for cucurbit viruses including two potyviruses, *Watermelon mosaic virus*, described in France since the early 70’s and *Zucchini yellow mosaic virus* that emerged world-wide in the 80’s and an ipomovirus, *Cucumber vein yellowing virus* that has been spreading in the Western Mediterranean basin in recent years.

## OP-50: Molecular epidemiology of *Watermelon mosaic virus* in France and evolution of viral populations

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*Watermelon mosaic virus* (WMV, Potyvirus) is known in France for more than 30 years, and is the most frequently detected virus in cucurbit crops. WMV has a larger host range than most potyviruses. In natural conditions, it infects many common weeds that represent potential virus reservoirs, and may allow local WMV isolates to persist in the environment in the absence of cucurbit crops. The agronomic impact of WMV in France remained limited for a long time, particularly on zucchini squash, since the virus induced only mild symptoms on leaves without any damages on fruits. Since 1999, more severe symptoms of leaf and fruit deformation, resulting in important agronomic losses, have been associated with WMV in zucchini squash crops. A molecular study revealed that only one group of strains was observed in France before 1999, whereas since 1999 a second group, highly divergent at the molecular level, was also detected. We performed epidemiological surveys from 2004 to 2007 in order to study the variability of WMV and its geographic structure. Within the "emerging" group, four molecular subgroups were defined unambiguously. Our results revealed a strong geographic structure of the classical and emerging isolates: whereas the classical isolates are found wherever WMV is present, the emerging ones are restricted so far to South-Eastern France. Besides, a structure was also observed at a lower scale between the subgroups of emerging isolates. In South-Eastern France, both types of strains are present, sometimes in the same field or the same plant, what raises the question of the interactions between classical and emerging isolates (competition between isolates, population replacements, and risk for emergence of recombinants with unknown biological properties...). This situation remained rather stable between 2004 and 2006, although the proportion of emerging isolates tended to increase. Our results suggest that the emerging strains spread only slowly from their site(s) of introduction but may progressively replace local strains.

## OP-51: The molecular basis of *Zucchini yellow mosaic* virus symptom expression

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*Zucchini yellow mosaic virus* (ZYMV) causes stunting, severe foliar deformation, blisters and mosaics in cucurbit species, eventually developing a filamentous leaf phenotype. The Helper-Component proteinase (HCPro) encoded by potyviruses is a multifunctional protein and its function as a suppressor of gene-silencing permits virus replication and movement. A point mutation in the conserved FRNK motif of the HCPro, from FRNK to FINK, dramatically reduces symptom expression without reducing the viability of the virus. Cucurbit plants inoculated with this attenuated virus do not exhibit leaf deformation and appear similar to healthy plants. Moreover, transgenic cucumber plants expressing ZYMV HCPro$^{FRNK}$ or HCPro$^{FINK}$ mimic the severe and attenuated ZYMV symptoms, respectively. Both wild-type ZYMV HC-Pro$^{FRNK}$ and attenuated ZYMV HC-Pro$^{FINK}$ are effective as suppressors of gene-silencing when expressed via *Agrobacterium* in a GFP-silencing suppression assay. Shortly after inoculation and before symptom appearance, some differences in miRNA accumulation levels, and dramatic differences in miRNA* levels, were detected between plants infected with the severe ZYMV HCPro$^{FRNK}$, compared to plants infected with the attenuated ZYMV HCPro$^{FINK}$. Additionally, the wild type HCPro$^{FRNK}$ specifically bounds siRNA-like and miRNA/miRNA*-like duplexes in an *in vitro* assay with a much higher affinity than the mutated HC-Pro$^{FINK}$. Additional mutations created in the FRNK box and its vicinity showed that the positively charged amino acid residues play a role in symptom severity. We demonstrated that several attenuated mutants retain their suppressor function while losing the ability to bind a siRNA-like duplex efficiently. Our data suggests that the highly conserved FRNK motif in the HC-Pro of potyviruses is a probable contact point with siRNA and miRNA duplexes. Possibly, the interaction of the FRNK motif with miRNA duplexes directly influences miRNA accumulation thereby up-regulating gene activity and thus causing symptom development.

## OP-52: Strains of *Potato virus Y* in Dutch seed potato culture

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Over the recent years *Potato virus Y* (PVY) presents a growing problem in Dutch seed potato culture. In the years 2002 – 2004 approximately 10 – 15% of the seed potato lots was de-classified because of the levels of PVY infection exceeded quality standards. In 2006 an even higher percentage was reported. Aphid vectors form an important factor in the spread of PVY. However, the increasing levels of infections with PVY are in contrast to the decreasing numbers of aphids caught in the yellow water traps and high suction traps used to monitor aphid flights. In addition to the well-known PVY-N and PVY-O standard strains, several new strains of PVY have been reported of which PVY-NTN and PVY-N-Wilga are currently the best known. Both strains are described as genetic recombinants between PVY-N and PVY-O. The presence and possible spread over The Netherlands of these recombinant strains is not known yet. In July 2006 a survey for PVY strains was done on potato plant material grown in the control fields of the General Inspection Service NAK. This plant material is representative of Dutch seed potato stocks. In the control fields tubers, which were taken from stocks tested by ELISA in 2005, are grown to evaluate the earlier laboratory tests by visual inspection. In the survey over 120 samples showing distinct PVY symptoms were collected from different seed lots. Each sample was tested by ELISA using polyclonal antibodies for the presence of PVY and monoclonal antibodies to distinguish PVY-N from PVY-O/C. All ELISA positive samples were further tested for the presence of PVY-N, PVY-O, PVY-NTN and PVY-N-Wilga by two molecular methods and in addition inoculated on a set of indicator plants. Results show that a significant shift in PVY strains populations in the Netherlands has occurred and that PVY-NTN and PVY-N-Wilga are more spread then previously and generally assumed.

**Keynote presentation**

## OP-53: Emergence and reemergence plant viruses in India: impact and management options

**Anupam Varma**

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In recent years emergence and reemergence of plant viruses, particularly in the tropics and semi tropics, has resulted in enormous economic losses and in many cases threatened food and nutrition security. These geographical regions provide ideal conditions for the perpetuation of both the viruses and their vectors. India, with its diverse agro-climate zones, is endowed with a large number of viral diseases affecting various cropping systems, but the diseases caused by the emerging and reemerging badna-, cucumo-, gemini-, ilar-, nano-, poty-, and tospoviruses are most threatening. Management of these diseases is difficult due to complex disease cycle, efficient vector transmission in most cases, and non-availability of effective viricide. Integration of various approaches like the avoidance of sources of infection, control of vectors, cultural practices and use of resistant host plants have been commonly used for the management of viral diseases of plants in India. Of these, use of resistant varieties has been most efective. However, rapid development of resistant breaking strains of viruses and lack of sources of resistance make breeding of resistant varieties difficult. Genetic engineering has provided new tools for developing crop varieties with durable resistance by 'pyramiding' genetically engineered resistance over intrinsic plant resistance. Efforts are being made in India to develop virus resistant transgenic plants (VRTPs) to manage economically important viral diseases of a variety of agricultural and horticultural crops. Horticultural crops cover nearly 17 million hectares with an annual production of about 150 million tonnes, contributing nearly 10% of world's fruit production and 11.4% of vegetable production. With the emergence of high-tech protected horticulture practices, floriculture is also catching up. But, viral diseases are a major constraint in improving the productivity of these crops. A major factor is the non-availability of certified quality seed/planting materials of horticultural crops, with the exception of potato. In the recent years tissue culture industry has also grown considerably for micro-propagation of more than 100 species of horticultural plants. To check spread of viral diseases through tissue culture raised plants, India has taken a lead by establishing a certification system of micro-propagated plants through a network facility for quality (genetic fidelity) and virus testing.

**Keynote presentation**

## OP-54: Variability is the arsenal that sustains war between plants and pathogens

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Diseases are caused by interactions between plants, pathogens and environment leading to epidemics and loss in the produce. Typically soil borne pathogens that are with a low multiplication rate and prolonged longevity have one infection cycle per season and cause monocyclic epidemics. Pathogens that are often airborne and capable of several infection cycles a season, cause polycyclic epidemics. The disease occurrence may encompass several growing seasons, especially if the crop is grown in monoculture year after year or as perennial crop. The inoculum produced in one season is carried over to the next leading to increases in inoculum load over the years. There are no clear breaks between growing seasons in the tropics unlike the forced breaks in temperate regions and this can lead to accumulation of inoculum. Yet, in any crop-pathogen encounters, the war is fought in astronomical numbers. Reaction of large populations of presumably different kinds of plants to a large population of pathogen under a range of macro- or micro-climate, nutritional and soil or water status can produce confusing set of observations on the level of resistance or susceptibility. Evidence on co-evolution of host and pathogen suggest for the presence of large variability in both host and pathogen. This variability must be dynamic i.e., continuously undergoing change, if the two organisms are to exist without eliminating each other. Artificial inoculations made on pure lines of a host plant species or cultivar with many isolates give different types of symptoms ranging from near immune to extreme susceptibility. This type of variable reactions has been reported in a number of host-pathogen combinations. Seldom is the degree of hereditary or genetic consistency known or static in individual pure lines of cultivars or isolates of the pathogens. This variability can further be influenced by environmental conditions. Among many plant pathogenic fungi, bacteria and viruses, innumerable new strains or races can result from mutation, and hybridization between biotypes within species, between species and between genera. Cultivated area of the crop, cultivar diversity deployed and the mode of reproduction in both crop and pathogen species as sexual and asexual or vegetative can further confound the level of observed variability. Strains of pathogen compatible to individual host-plant resistance genes are always present in the environment at detectable frequencies. Both the loss in virulence of a pathogen population and its spontaneous reversion occur frequently in nature. Genotypes showing resistance in the country of origin are known to be susceptible elsewhere. Investigations on the variability in the host and pathogen help discover and pool resistance genes. Pathologists continue to assess in limited experiments or in multi-environment tests (METs) the importance of such changes in fungus, bacterium and virus pathogens so that with this information new cultivars possessing adequate resistance may be developed. Results from investigations on several pathosystems demonstrate variability as a dynamic weapon for the survival in the combatants in the environment. This variability can be equally used as a tool for humans to tilt the balance in the contest to favour the crop species. Based on such an understanding, pathologists and breeders used METs to successfully develop many varieties with durable resistance to diseases. Genomes sequence comparisons and functional analysis underway will allow to dissect variability and discern specific genes and quantitative resistance loci at molecular level. In future, the knowledge generated in molecular biology will also be evaluated through METs prior to integration for commercial use.

## OP-55: A diversity study of cassava brown streak virus(es) infecting cassava in East Africa

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The Ipomovirus, *Cassava brown streak virus* (CBSV) is the causal agent of the brown streak disease that presents a serious impediment to cassava production in Eastern African countries. Information on the virus and on disease parameters is limited due to the enigmatic nature of the virus. Hence, CBSV was isolated from CBSV/ EACMV mixed infected cassava plants collected from coastal Kenya in 1999. Back transmission to several IITA cassava breeding lines resulted in single CBSV infections with pronounced symptoms and providing proof of the Koch's postulates. Several Kenyan virus isolates collected at different sites were subjected to molecular and biological studies that included inoculation/ infection experiments for varietal responses, virus resistance, synergism with cassava geminiviruses and, insect transmission studies. The molecular analysis of complete virus CBSV ssRNA genomes revealed unique genome features and provided the basis for sequence analysis of several CBSV isolates from Mozambique, Malawi and Uganda. This comprehensive analysis of CBSV isolates from East African cassava with brown streak disease, presents a framework for the description of this virus. Molecular diversity and biological features of CBSV will be discussed with respect to disease development in Cassava and the definition of strategies for virus resistance.

## OP-56: *Honeysuckle yellow vein mosaic* and *Tobacco leaf curl Japan viruses* with or without DNAß satellites produce yellow dwarf disease of tomato in Japan

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Two begomoviruses (Nara virus-1 and Nara virus-2) and a satellite DNA (DNAβ-Nara) were cloned from Honeysuckle (*Lonicera japonica*) infected plants exhibiting characteristic yellow vein mosaic symptoms. A satellite DNA (DNAβ-Ibaraki) associated with Ibaraki begomovirus, which induces typical yellow net symptoms and small elliptical enations on the abaxial side of the leaves, was cloned from Honeysuckle plants. Comparison of the nucleotide sequences with other begomoviruses revealed that Nara virus-1 and Nara virus-2 were strains of the *Honeysuckle yellow vein mosaic virus* (HYVMV), hence they were named as HYVMV-[Nara1] and HYVMV-[Nara2]. Whereas Ibaraki virus was a strain of *Tobacco leaf curl Japan virus* (TbLCJV), which we designated as TbLCJV-Hs[Iba]. HYVMV-[Nara1] and HYVMV-[Nara2] has hybrid genomes which arose from recombination among HYVMV and TbLCJV. On tomato, TbLCJV-Hs[Iba] or HYVMV-[Nara2] alone produce yellowing, leaf curling and stunting symptoms. When associated with DNAß, symptoms were severe and incubation period was shortened. We concluded that HYVMV and HYVMV / DNAβ as well as TbLCJV-Hs[Iba] and TbLCJV-Hs[Iba] / DNAβ were causal agents of tomato yellow dwarf disease. Honeysuckle is a potential reservoir host for geminiviruses that causes tomato yellow dwarf disease.

## OP-57: Toward identification of grapevine-infecting viruses in vineyards of Iran-*Grapevine fanleaf virus* isolates

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North-west Iran and Eastern Anatolia is the origin of grapevine. This crop has been cultivated in Iran for thousands of years, however almost no attention therein has been paid to the virus infection status of the vines. After setting up a plant virology lab at the University of Tabriz we have now begun to survey the vineyards for presence of *Grapevine fanleaf virus* (GFLV) at first place because of its worldwide distribution. We have been inspecting vineyards in East and West Azarbaijan and Ardabil provinces in the northwest region since 2003. Leaf samples, expressing virus-like symptoms, were collected from the vineyards and subjected, first, to DAS-ELISA with anti-GFLV antibody. As a result, infection with GFLV was proved in many samples although they formed a relatively small proportion of the collected samples. Then, an RT-PCR assay was adapted to identify the virus isolates at molecular level and determine the virus genetic diversity. Accordingly, total RNA from the ELISA-positive leaves was subjected to first strand cDNA synthesis with oligo $d(T)_{16}$ and followed by PCR with the previously designed primers corresponding to the virus coat protein (CP) and movement protein (MP). Fragments with the expected sizes, amplified from many of the isolates, were cloned in a T/A cloning plasmid and subjected to sequencing. When the sequence data were subjected to phylogenetic analysis, a wide diversity (up to 17%) was revealed between the isolates at both CP and MP regions. Interestingly, some isolates appeared to be distinct among all the GFLV isolates recorded in the databases suggesting that they have probably originated from Iran. That a great majority of the ELISA-positive samples gave no amplification in the PCR suggested two possibilities. First, the primers used in this research were designed according to the GFLV isolates from other parts of the world and accordingly they might not anneal to the isolates from Iran. This is quite possible if the highly variable nature of the virus is taken into account. Second, there might be other virus species infecting the vines and causing symptoms similar to those incited by GFLV. This is also possible because about 60 virus species have been reported from grapevine. Having now fragments of the local GFLV isolates sequenced, we have designed primers accordingly that may increase possibility of detecting the isolates with a higher efficiency.

## OP-58: Characterization of a monopartite recombinant begomovirus and satellite DNAβ associated with yellow vein mosaic disease of mesta crop in India

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Mesta (*Hibiscus cannabinus* and *Hibiscus sabdariffa*), a bast fibre crop, suffers from yellow vein mosaic disease that appears for the last three years in endemic form in different mesta growing areas in India. Surveys revealed 90% incidence of the disease and the yield loss due to this disease alone was found to be 12.78 - 17.45% with respect to fibre yield and 18.91-23.83 % with respect to seed yield. Transmission of the disease by whitefly (*Bemisia tabaci*) and association of geminate particles as revealed through electron microscopy indicated the presence of begomovirus in the symptomatic samples. Positive hybridization signal in Southern blot using radio-labelled probes to *Cotton leaf curl Rajasthan virus* DNA-A and β–DNA confirmed the involvement of a begomovirus with the disease. Coat protein gene of associated begomovirus and the satellite DNAβ molecule from six different geographical isolates were amplified using specific primers, cloned and sequenced. However, none of the DNA-B specific primers used could generate any amplicons and thus indicated the monopartite nature of the begomovirus under study. From the coat protein gene sequences an abutting primer set was designed which could amplify the full length DNA-A homologue (~2.7 kb) of the begomovirus. Sequencing of this clone (EF428256) revealed that it consisted of 2752 nt. and shared highest (84.3%) sequence identity with *Cotton leaf curl Bangalore virus* followed by *Malvestrum yellow vein virus* from china. It has been noticed that this DNA-A molecule shared less than 89% sequence identity with any known begomovirus sequence and hence it is proposed as a new species of begomovirus with a tentative name *Mesta yellow vein mosaic virus* (MeYVMV). Full length DNA A has two functional open reading frames (ORF) in viral sense and five ORFs in complementary sense. Individual ORF analysis revealed that this DNA-A evolved as a recombinant molecule from atleast three distinct begomoviruses from north India, south India and China. The phylogenetic tree derived from full length DNA-A showed that MeYVMV did not group with any begomovirus isolate. The genome organization of DNAβ molecules characterized from north and East Indian isolates were found to be similar as other DNAβ but the East Indian isolates shared more similarity with cotton leaf curl DNAβ and separated out from the cluster containing the north Indian isolates. The phylogenetic tree derived from the coat protein sequence of 6 isolates of *Mesta yellow vein mosaic virus* with that of other different begomovirus isolates showed that the MeYVMV isolates obtained from eastern and northern India formed two distinct groups indicating the existence of two distinct begomovirus complexes associated with the yellow vein mosaic disease of mesta in two different geographical location in India. Nucleic acid based diagnostics have been developed for identification of these complexes. This study, although established the occurrence of a new begomovirus DNAβ molecule with mesta yellow vein mosaic disease, yet agroinoculation with the complex is necessary to establish its causal relationship.

# *Abstracts of Poster Presentations*

# Poster Session – I

## PP-1_01: Rhizomania in Iran, a disease under strong selection pressure

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Sugar beet is a major crop in Iran with now nearly 160,000 ha under cultivation. The rhizomania disease of sugar beet is now present in almost all of major sugar beet growing areas within the country. In order to have a clear view over rhizomania situation in Iran, a comprehensive study was conducted. Up to 300 soils and sugar beet samples were collected throughout the country in 2006, using a smart sampling procedure. Out of these 300 samples, 203 proved to be infected by the *Beet necrotic yellow vein virus* (BNYVV), the causal agent of rhizomania, representing thus 66% of the sugar beet fields sampled. Two different virus types were found by targeted sequence analysis. The RNA-3 p25 gene, involved in the virulence of BNYVV, was then systematically sequenced, with the view of characterizing the virus diversity throughout the country. Seven different variants of the p25 were found in Iran. Most of the Iranian infected samples were related to BNYVV type A, with six different aa tetrads AHHG, ACHG, AYHG, ALHG, AFHR, AFHG and just one in BNYVV type P with a SYHG tetrad. Only 18 mutations at nucleotide level were recorded for the RNA-3 p25 660 nucleotide fragment, resulting in 15 amino acid changes. Such a high ratio of aa changes indicates a strong pressure on the virus, may be due to the recent introduction of tolerant sugar beet cultivars combined to high inoculum potential in Iranian *Polymyxa betae*-infested soils. Concurrently, soils were also checked for the presence of BSBV and BVQ, two soil-borne pomoviruses often associated with BNYVV. In total, 96 samples out of the 203 BNYVV positive isolates (47,3%) were found positive for the presence of BSBV and 30 (14,7%) for BVQ. Strategies for the management of the viral disease in Iran will be discussed

## PP-1_02: Development of post-transcriptional gene silencing (PTGS) constructs against *Groundnut bud necrosis virus*

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Groundnut bud necrosis caused by *Groundnut bud necrosis virus* (GBNV), an enveloped RNA virus transmitted by thrips, is remerging as most economically important virus disease on several crops in India. In the present study, an effort was made to develop various constructs for inducing post-transcriptional gene silencing (PTGS) of GBNV in host plants. The nucleoprotein (*NP*) gene sequence of different GBNV isolates was downloaded from public databases, aligned and oligonucleotide primers were designed to amplify *NP* gene from 415 to 769 bp. Virus-infected groundnut leaf (*Arachis hypogae* L.) samples were collected from three districts of Karnataka and partial *NP* gene region between 415 to 769 bases was amplified cloned and sequenced. The clones with viral gene inserts were PCR amplified and restricted with endonuclease enzymes, *Bam HI* and *KpnI* for antisense, *NcoI* and *XhoI* for sense cloning into generic intron hairpin (ihp) and *NcoI* and *XhoI* for cloning into short heterologous 3'UTR (SHUTR) vectors. The generic ihp vector has a functional CAT intron of castor (*Ricinus sativus*) downstream of CaMV 35S promoter and flanking either side by multiple cloning sites to facilitate directional cloning in sense, antisense and in both orientations. For generic SHUTR construction, functional catalase (CAT) intron was cloned downstream of CaMV 35S promoter, with poly (A) inserted on both sense and antisense orientation to give hairpin structure of the 3' UTR region. The dsRNA resulting from the inverted polyadenylation signal in the 3' region of the transcript trigger RNA degradation that include not only the upstream transgene but also sufficient to induce sequence specific degradation of endogenous mRNA homologous to the transgene targeted sequence. Partial *NP* gene sequence of GBNV was cloned in sense, antisense and both (sense/antisense) orientation in generic ihp vector to develop three PTGS constructs and in SHUTR vector to develop SHUTR construct against GBNV. The confirmed trasformants of all the four constructs were sub-cloned into pCAMBIA 1305.1 promoter less plant transformation vector and the confirmed clones were mobilized into *Agrobacterium tumefaciens* LBA 4404 with *E.coli* pPRK2013 as helper. These constructs are being used for transformation of tobacco (*Nicotiana tabacum* var. White pearl) validate the efficacy of each of the vector in inducing PTGS against GBNV.

## PP-1_03: Host range of *Beet curly top virus* (BCTV) in Khorasan and Hamadan provinces in Iran

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*Beet curly top virus* (BCTV) is a single – stranded DNA virus belonging to the family Geminiviridae and it is the type member of *Curtovirus* genus. In order to investigate curly top virus occurrence in commonly occurring weeds in sugar beet cropping system, a survey was conducted during 2003 – 2006 in Iran. Weekly samplings were carried out in four major areas of sugar beet cropping fields in Khorasan and Hamadan. Samples were collected from plants showing disease symptoms such as leaf curling, venial chlorosis and enations, and transferred to laboratory to identify causative virus. PCR was conducted for virus detection and virus preparation was inoculated into healthy beets by adopting preparation injection. *Amaranthus retreflexus, Chenopodium album, Malva sp*. and *Convolvolus arvensis* were recognized as BCTV hosts in the fields. The virus in weeds was observed almost in all sugar beet cropping areas of Hamadan and Khorasan provinces. However, incidence was higher and symptoms were clear in warm areas.

## PP-1_04: Experiments on tuber necrotic ringspot isolates of *Potato virus Y* (PVY$^{NTN}$) in tomato in Hungary

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*Potato virus Y* is the type member of the family *Potyviridae*, which constitutes the largest known and economically most important family of plant viruses. Potato tuber necrotic ringspot disease (PTNRD) is caused by the NTN strain of *Potato virus Y* (PVY$^{NTN}$), which produces severe necrotic ring symptoms on the tubers and berries of potato. The disease was first described in Hungary and subsequently spread to other parts of the potato growing regions in the world. PVY is the most common viral pathogen in potato and also in tomato crop in Hungary. The nucleotide sequence of the coat protein (CP) gene and the 3' non-translated region of 3 PVY isolates were studied. The isolates were collected from tomato fields in different regions of Hungary. *N. tabacum* cv. 'Xanthi-nc' plants were mechanically inoculated with PVY isolates. Total nucleic acids were extracted from systemically infected leaves. cDNA was prepared using poly T primer. The coat protein gene and the 3'NTR region were amplified by RT-PCR using degenerate potyvirus primers 7941 [5'-GGA ATT CCC GCG G (AGCT25-25%) AA(CT50-50%) AA(CT50-50%)AG(CT50-50%) GG(AGCT25-25%) CA(AG50-50%) CC-3'] and a poly $T_2$ (5'-CGG GGA TCC TCG AGA AGC TTT TTT TTT TTT TTT TT-3'). The amplification was carried out in 40 cycles (one cycle consisted of 94°C for 15 seconds, 45°C annealing for 30 seconds, 72°C extensions for 3 minutes). Sequence analyses were performed using the University of Wisconsin Genetics Computer (GCG) sequence analysis software package version 9.1. Sequence comparisons were performed with EMBL/GenBank databases, and the phylogenetic tree was constructed using the Drawtree software. Nucleotide sequences of the CP region and 3' non translated region (NTR) were determined. Sequence data were sent to the EMBL GeneBank Database (AM746613, AM746614 and AM746615). Sequences were compared with PVY$^{NTN}$ isolates from Hungarian potato. Homology of nucleotide and amino acid sequences were high among the studied PVY isolates. According to the characteristic regions, all isolates belonged to the PVY$^{NTN}$ strain. It is concluded that NTN strain of PVY, also distributed in tomato, play an important role in the epidemiology of solanaceous plants.

## PP-1_05: Pre-exposure of calli to ozone promotes systemic resistance of the regenerated *Capsicum annuum* cv. PKM1 (Chili) plantlets against *Cucumber mosaic virus* attack

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Studies were undertaken to evaluate ozone ($O_3$) for induction of systemic resistance against *Cucumber mosaic virus* (CMV) in *Capsicum annum* cv. PKM1 (chili) plantlets. Calli was induced from chili leaf explants on Murashige & Skoog's (MS) medium supplemented with 9.05 μM 2,4-dichlorophenoxy acetic acid (2,4-D). The 30 days old well developed calli induced from tomato leaf explants were subjected to pre-treatment with ozone, i.e. $T_1$ =100 ppb, $T_2$ = 200 ppb, $T_3$ = 300 ppb for a period of 30 min repeatedly for 7 days and for control (C) calli, charcoal filtered air was supplied. Regeneration of shoots was obtained by culturing ozone treated calli on MS medium containing 4.4 μM benzyladenine and 2.9 μM gibberalic acid. The frequency of regeneration of tomato plantlets from the calli were $T_1$ = 84%, $T_2$ = 72%, $T_3$ = 56%, but for control 93% regeneration was obtained. Regenerated plantlets were rooted in half strength MS medium supplemented with 2.9 μM Indole-3-acetic acid and successfully acclimatized. The plants regenerated from ozone treated calli are referred to as $T_1$, $T_2$ and $T_3$ plants, which hold remarkably increased soluble phenolic content compared to the control plantlets. All the plantlets were challenge inoculated with CMV and shows disease incidence ranged from $T_1$ = 27%, $T_2$ = 38%, $T_3$ = 47% and C = 91%. Remarkable increase in activities of salicylic acid (SA), phenylalanine ammonia-lyase (PAL) and peroxidase (POX) were detected after *Cucumber mosaic virus* inoculation, in foliar extracts of $T_1$ plantlets than $T_2$ and $T_3$, compared to the control plantlets. Therefore, plantlets regenerated from 100 ppb ozone treated calli shows enhanced systemic resistance against CMV attack.

## PP-1_06: *Banana bunchy top virus*: an increasing threat to banana production in Pakistan

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Banana bunchy top disease (BBTD) is the most important and serious virus disease of banana all over the world. BBTD causes severe damage and losses to the production of banana in many countries and is a major constraint to the production of banana in many areas of South East Asia and pacific. It became a major threat in Pakistan in late 80's in the area of Sindh province, which is the main area in Pakistan for the production of banana. The disease is caused by a single-stranded circular DNA virus known as *Banana bunchy top virus* (BBTV), which is a member of the genus *Babuvirus* (family *Nanoviridae)*. Nanoviruses have multipartite genomes, each component being approximately 1 kb in size. BBTV is transmitted both through vegetative propagation of affected plants and by the aphid vector *Pentalonia nigronervosa*. In the present study we carried out the cloning and characterization of the genomic components of BBTV from Pakistan. Sequence analysis and phylogenetic studies were performed for all BBTV components. We have designed primers to a highly conserved region of master replication associated protein that are useful for polymerase chain based detection of BBTV in banana. In addition, primers to banana genomic sequence are used as an internal control. Together these primer sets are a valuable tool in the effort to control BBTV, particularly in screening micropropagated banana plantlets for the absence of virus before release to farmers.

## PP-1_07: Weeds as a source for inoculum of viruses and potential threat to cultivated crops

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Several weeds, including *Sonchus arvensis*, *Digera arvensis, Conyza stricta, Croton glandulosus* and *Cichorium intybus* are commonly found around cultivated fields and exhibit vein yellowing and yellow mosaic symptoms. *Coriopsis tinctoria,* a common horticultural plant shows similar symptoms. Potato and a common weed, *Xanthium strumarium,* show symptoms typical of begomoviruses (including leaf curling and vein thickening). Total DNA isolated from such infected plants and universal primers for begomovirus DNA A and begomovirus-associated DNA ß were used to amplify these components. Fragments of 2.8 kb and 1.4 kb, respectively, were amplified from each plant, cloned and are in the process of being completely sequenced. In control experiments the same primers failed to amplify any fragments from total DNA isolated from healthy plants. The presence of begomoviruses was also confirmed by Southern blot analysis. These results show that *Digera arvensis, Conyza stricta, Cichorium intybus, Coriopsis tinctoria* and also potato harbour previously uncharacterized geminivirus. Since weeds are believed to act as reservoirs of viruses for infecting crops, characterization of these is important to assess the likely threat they pose to agriculture. We have sequenced DNA A and associated DNA ß satellite molecule from all these weeds. The presence of multiple and recombinant DNA ß in these weed hosts indicate that weeds are an important source of multiple infections and likely play a role in generation of recombinant molecules that may enhance their host range. Additionally, no commercially important potato infecting geminiviruses have yet been identified in Pakistan and the etiology of the disease in potato needs to be clarified with a view to establishing the potential threat of this virus to potato production in the country.

## PP-1_08: First report of *Tobacco streak virus* on *Cosmos bipinnatus* Cav. and *Impatiens balsamina* L. from India

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During 2006, cosmos (*Cosmos bipinnatus* Cav.) and impatiens (*Impatiens balsamina* L.) plants from Dawalwadi, Jalna, Maharashtra state, India showed characteristic symptoms of necrosis. Field infected cosmos plants showed necrosis of leaves, petiole, stem and apical buds, and severe deformation of the young leaves Infected impatiens plants showed necrosis of leaves, petiole and stem, and with necrotic oak leaf like pattern on the young leaves. Field collected leaf samples were tested by ELISA (enzyme linked immunosorbant assay) using antisera specific to *Tobacco streak virus* (TSV; Mahyco Research Center), *Groundnut bud necrosis virus* (provided by Dr. D.V.R. Reddy), *Tomato spotted wilt virus* (DSMZ AS-0105), *Impatiens necrotic spot virus* (DSMZ AS-0115), *Chrysanthemum stem necrosis virus* (DSMZ AS-0529), *Iris yellow spot virus* (DSMZ AS-0528), *Papaya ringspot virus* (AS-0805) and *Zucchini yellow mosaic virus* (AS-0234). Symptomatic leaves specifically reacted to TSV antiserum, but failed to react with any of the other antisera tested. Sap from symptomatic leaves was used to inoculate indicator host species by mechanical inoculations. The inoculations resulted in local necrotic lesions on *Vigna unguiculata* cv. C-152 (cowpea) and *Nicotiana tabacum* cv. Xanthi (tobacco) at 2 to 3 days post inoculation (dpi) and systemic necrosis in cowpea and necrosis with oak leaf like pattern in tobacco at 7 to 9 dpi. Total RNA was isolated from the virus inoculated cowpea leaves and used for RT-PCR using TSV coat protein (CP) specific primers. A ~720 bp RT-PCR product was cloned in pGEM-T vector and sequenced. Sequence analysis of TSV-cosmos and TSV-impatiens shared 99.1 -99.4% and 98.7- 99.5% identities at nucleotide and amino acid levels, respectively, with TSV Jalna isolate (AY 505073). Based on the symptoms, virus transmission, serology and CP gene identity, the causal agent for necrosis of cosmos and impatiens is confirmed as strains of TSV. This is the first report of TSV in cosmos and impatiens from India.

## PP-1_09: Tomato torrado virus - new virus transmitted by greenhouse whitefly (*Trialeurodes vaporariorum*) in Poland

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Of about 115 virus species transmitted by whiteflies, 90% belong to the *Begamovirus* genus, 6% to the *Crinivirus* genus and the remaining 4% are classified to *Clostervirus, Impovirus and Carlavirus.* In Poland, only *Trialurodes vaporariorum* occurs. Greenhouse whitefly is a vector of four species of *Crinivirus* genus. However, until now no virus was found to be transmitted by *T. vaporariorum* in Poland. In 2003, a spherical virus was isolated from tomato cv. Grace that showed stunting, chlorosis and necrosis of leaves. Occurrence of the virus was strictly associated with presence of *T. vaporariorum.* Elimination of the greenhouse whitefly resulted in elimination of the virus. The virus was vectored by *T. vaporariorum* very efficiently (100%) and transmitted mechanically with difficulty (50-70%). After mechanical inoculation or whitefly transmission, the virus caused systemic infection on *Nicotiana tabacum* (cvs. Xanthi nc, Samsun, White Burley) *N. benthamiana, N. clevelandii, N. debneyi, N. affinis, N. glutinosa, Lycopersicon esculentum, Petunia hybrida, Capsicum annuum, Nicandra physaloides, Physalis floridana and Solanum tuberosum*. The virus did not infect: *Phaseolus vulgaris*, *Pisum sativum*, *Cucumis sativus*, *Chenopodium quinoa* and *Beta vulgaris.* Symptoms on *L. esculentum* plants varied on different tomato plants varieties. Some of them expressed severe symptoms including stunting, deformations and necrosis while on others varieties milder symptoms including mosaic necrotic lesions or lack of symptoms were observed. An electron microscopy examination of the sap from infected plants showed the presence of spherical virus particles of 25 nm in diameter. Purified virus preparation centrifuged in the density sucrose gradient sedimented as two separated zones. The viral genome was divided into two RNA molecules of about 7500 bp (RNA1) and 5500 bp (RNA2). The capsid consisted of three proteins. In an immunoelectron microscopy the Polish isolate reacted only with antiserum against *Tomato necrotic dwarf virus* (ToNDV). ToNDV was isolated and partially characterized in 1984, in California, USA. ToNDV possessed some properties (the symptoms on tomato, the morphology of particles, transmission by whitefly *Bemisia tabaci*) similar to the Polish *T. vaporariorum* transmitted virus. The new Tomato torrado virus (ToTV), isolated in Spain, showed the same particles, genome organization and biological properties as the Polish isolate. Based on the full length genome of ToTV (Accession No. DQ388879 and DQ388880), we designed two pairs of specific primers for reverse transcription polymerase chain reaction (RT-PCR), which amplified the fragments of about 892 bp for RNA1 and 573 bp for RNA2, respectively. RT-PCR products were sequenced and the obtained sequences have been deposited in the GenBank database under the accession numbers EF6350007 and EF635008. The sequences' comparison between the Polish isolate and ToTV showed 99 and 98% homology for RNA1 and RNA2, respectively. The similarity of tomato plants symptoms, virus particles morphology, genome organization and nucleotide sequence identities suggest that the Polish *T. vaporariorum* transmitted virus and ToTV are the same.

## PP-1_10: Incidence and diversity analysis of *Prunus necrotic ring spot virus* infecting *Prunus* sp.in the North-Western Himalayas, India

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*Prunus necrotic ring spot virus* (PRSV) is a member of the Ilarvirus genera, family bromoviridae. Genus Ilarvirus comprises large group of plant viruses infecting primarily woody hosts especially *Prunus* spp. (stone fruits). Stone fruits (plum, peach, cherry, apricot and almond) and pome fruits (apple and pear) are the major fruits grown in the North western Himalayan region on commercial scale. These fruits are mainly grown in the states of Himachal, Uttaranchal and Kashmir. Threat to their production is due to many fungal and viral pathogens, which lower their quality and quantity. In the present study several surveys were conducted in the various stone and pome fruit growing areas of Himachal Pradesh (Kullu, Solan, Palampur, Chamba and Sirmour areas). Samples wee collected from trees showing shot hole symptoms and they were analyzed by enzyme-linked immunosorbant assay (ELISA) using PNRSV specific antibodies (Agdia, USA). Positive results were obtained from plum, peach, nectarine, cherry and almond including Himalayan wild cherry (*Prunus cerosoides*). To confirm PNRSV, RT-PCR was performed using specific primers for the coat protein gene. Fragment of about 675bp was amplified as reported. Sequencing results confirmed the presence of PNRSV in the plum, peach, nectarine, wild cherry and almond (accession numbers: AM494934, AM408910, AM408909, AM493717 and AM712614). These were compared with 14 isolates from other countries using ClustalW programme. The isolates showed 87% to 100 % homology among each other at nucleotide and amino acid level. Indian isolate of PNRSV infecting stone fruits was classified in group PV-96 on the basis of amino acid variability. Earlier PNRSV infecting ornamentals, especially rose and pelargonim (acc.no. AJ619958, AJ969110) were classified in Group PV-32 on the basis of amino acid variability. The cloned fragment has been used as probe for PNRSV during nursery certification and disease monitoring. This will be the first step towards effective management of PNRSV through virus-free propagative material.

## PP-1_11: Studies on symptomatology and host range of a virus causing necrotic wilt in sunflower

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Necrosis and yellowing diseases on sunflower are of common occurrence in Marathwada region during *kharif* and *rabi*. The disease with necrotic symptoms is predominant in *kharif* while yellowing is predominant in *rabi*. Virus isolates *viz.* isolated-N (I-N) and isolate-Y (I-Y) eluted from naturally infected sunflower plants showing necrosis and yellowing diseases respectively have been tentatively identified as two different strains of *Tobacco streak virus* on the basis of symptomatology, reaction on diagnostic host, transmission, physical properties and host range studies. Studies revealed that virus causing necrotic wilt in sunflower has symptoms like chlorotic specks, which developed into necrotic spots later on showing veinal necrosis. On stems, symptoms appeared in the form of light brown strips which later on turns dark brown and leads to stem necrosis. Severely infected leaves dried. Probably it is the first report stating a strain of TSV that can cause yellowing in the sunflower. The virus could infect and produce symptoms on eight out of nineteen plant species belonging to the chenopodiaceae, cucurbitaceae, leguminoseae and solanaceae.

## PP-1_12: Comparison of suppressiveness of *Cucumber mosaic virus* infection *by foliar spray of compost and vermicompost mixtures* in *Lycopersicon esculentum cv.* PKM1

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The degrees of suppression of *Cucumber mosaic virus* (CMV) infection in tomato seedlings by compost and vermicompost mixtures obtained from different animal wastes (cattle manure, poultry manure) and plant biomass (neem, pongamia etc.) were compared in greenhouse experiments. Compost and vermicompost mixtures were applied as foliar sprays and pot mixtures. The compost and vermicompost mixture as foliar spray minimized the CMV infection in tomato seedlings, whereas pot mixture was not effective in reducing the disease incidence. Together, the compost and vermicompost mixture help the plant to utilize the nutrient source and promotes growth of the plant but did not involved in the disease suppression, whereas foliar spray suppresses CMV infection.

## PP-1_13: Incidence of plant virus in Antarctica

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This work was focused on the assessment of plant virus spread among higher plants in Antarctic region. Sampling occurred during several seasons at the locality of Ukrainian Antarctic Station "Academician Vernadskiy". In our research we utilized ELISA for testing samples of two higher cereal plant species *Deschampsia antarctica* and *Colobanthus quitensis*, for presence of most common plant viruses. Surprisingly, the analysis demonstrated that samples of *D. antarctica* were found to be positive for viruses belonging to different taxonomic groups: *Cucumber green mottle mosaic virus* (*Tobamovirus*), *Cucumber mosaic virus* (*Cucumovirus*), and *Tomato spotted wilt virus* (*Tospovirus*). Samples of *C. quitensis* contained antigens of *Cucumber green mottle mosaic virus*. This data not only proves the unexpectedly high diversity of plant virus antigenic determinants being detected in Antarctica, but also raises a question of virus specificity and plant susceptibility, as normally these viruses infect dicotyledonous plants, not cereals. Further, these results were partially supported by bioassay and electronic microscopy data. However, means of plant viruses' emergence in the region remain elusive and are discussed in the work.

## PP-1_14: Spread of *Plum pox virus* in Ukraine

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*Plum pox virus* (PPV) belongs to Genus *Potyvirus,* infects almost all species of stone fruits and is the most harmful agent of viral etiology in plum, apricot and peach in Europe. Besides, PPV is considered as a quarantine virus all over the world. Taking into consideration the absence of data about of PPV occurrence in Ukraine, we have carried out monitoring of plantings of stone fruit cultures in different regions of Ukraine for PPV during 2004-07. We have carried out visual diagnostics of disease according to the symptoms typical for PPV in Transcarpathian, Kyiv, Odesa, Kharkiv and Khmelnytskiy regions. The symptoms presented as degraded rib spots or circles on the surface of leave blade that had light green or yellow-green color were observed mostly in Transcarpathian and Odesa regions. Samples of plum, apricot, peach and cherry-plum leaves containing symptoms typical for PPV, and without visible symptoms were selected in 5 regions and tested for the presence virus infection using enzyme-linked immunosorbent assay (ELISA) with commercial PPV antisera (Loewe Biochemica, Munich, Germany). While selecting the samples the highest attention was paid to Transcarpathian and Odesa regions as the symptoms typical for plum pox were observed in these regions. PPV was detected in all the surveyed regions, but percent incidence varied in each region. In Transcarpathian region PPV was found in 47.5% of tested plants, and virus was identified in 6 districts-Berehivskiy, Vynogradivsky, Irshavskiy, Mukachivskiy, Uzhgorodskiy and Khustskiy. In Kyiv region, PPV incidence was 13%, and the virus was detected only in Kyivo-Svyatoshin district, while in Vasylkivskiy and Myronivskiy districts PPV was not found. While testing the samples selected from 2 districts of Odesa region PPV was detected in 32% of explored samples, and it is curious that in Bilyaiv district the percent of infected trees included 65.6%, and in Ovidiopil district it was only 15%. For the first time occurrence of PPV in Kharkiv and Khmelnytsk regions was shown. Results obtained using ELISA match the data of visual diagnostics of plantings as well as data of electron microscopy study which revealed flexuous filamentous particles with 760 nm in length and 20 nm in diameter.

## PP-1_15: Natural occurrence of *Dasheen mosaic virus* in aroid tuber crops and foliage ornamentals in Andhra Pradesh, India

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Taro [*Colocasia esculenta* (L.) Schott] and elephant foot yam (*Amorphophallus paeoniifolius*) are grown in the humid tropical and sub-tropical environments for their tubers as a source of staple food. Aroids like *Spathiphyllum, Alpinium, Aglaonema, Caladium, Dieffenbachia, Anthurium, Syngonia* and *Philodendron* are widely grown both as an indoor and outdoor foliage ornamentals. Aroid plants are propagated through vegetative propagules (stem pieces, tubers, corms) and thus viruses infecting them are vertically transmitted and safely perpetuated in nature. Viruses like *Dasheen mosaic virus* (DsMV), *Colocasia bobone disease virus* (CBDV), *Tomato spotted wilt virus* (TSWV), *Banana bunchy top virus* (BBTV) and *Tobacco necrosis virus* (TNV) were reported to naturally infect aroid plants in different parts of the world. DsMV has a worldwide distribution. Its incidence is highly variable on different plants and regions affecting the growth of the aroid plants and is considered a quarantine threat. Aroid tuber crops and foliage ornamentals were surveyed in East and West Godavari, Krishana and Chittoor districts, Andhra Pradesh for DsMV infections during January to July, 2007. DsMV infections were confirmed by checking representative samples by ELISA using *Tobacco etch virus* antiserum. Incidence and symptomatology varied with plant species in taro and elephant foot yam fields. DsMV incidence ranged from 10 to 52% in Taro (feathery mottle and ring spots), 1 to 60% in elephant foot yam (mosaic and leaf mottling), 0.6 to 100% in *Spathiphyllum* sp. (leaf deformation, white streaks, leaf stunting), 0.5 to 4% in *Alpinium* sp (leaf distortion, chlorosis,), 0.3 to 59% in *Aglaonema* sp (leaf mottling), 1 to 12% in *Caladium* sp (leaf deformation), 0 to 7% in *Dieffenbachia* sp (leaf mosaic, ring spots, distortion and stunting), 0 to 22% in *Anthurium* sp (leaf deformation, chlorotic bands along midrib or between veins), 0 to 10% in *Syngonia* sp (marginal mottling) and 40-50% in *Philodendron* sp (leaf mosaic, chlorotic streaking along veins and leaf distortion). A few asymptomatic plant species were also found to be positive in ELISA, indicating the higher incidence of DsMV. Further work is in progress on molecular epidemiology of DsMV isolates.

## PP-1_16: Biological and molecular characterization of *Pea seed-born mosaic virus* of important faba bean fields of Iran

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Cultivated faba bean is used as human food in developing countries. It can be used as a vegetable, green or dried, fresh or canned. It has been cultivated in different provinces of Iran. Viral diseases cause a major constraint to faba bean production and can lead to a significant reduction in yield and seed quality. Several viruses' cause diseases in faba bean including *Bean yellow mosaic virus (*BYMV*), Bean leaf roll virus* (BLRV), *Broad bean mottle virus* (BbMV*) and Pea seed born mosaic Virus* (PSbMV). *Pea seed born mosaic virus* (PSbMV), of the genus *Potyvirus* is present in most of the faba bean plants. A survey was conducted during growing seasons of 2005 and 2006 to identify viruses infecting faba bean (*Vicia faba* L.) in four provinces (Khuzestan, Golestan, Isfahan and Tehran) of Iran. A total of 331 faba bean samples with symptoms of viral infection (leaf rolling, yellowing, plant stunting, small seeding and malformation of leaves) were collected. The samples were tested with serological methods (ACP-ELISA, DAS-ELISA, Dot blot immunobinding assay and Tissu printing immunoassay) with specific antisera for PSbMV and Potyviruses and also using IC-RT-PCR (Immunocapture-Reverse Transcriptase-Polymerase Chain Reaction) with specific primer pairs of coat protein region of PSbMV for all isolates. Most of the samples were infected by PSbMV. Among 331 infected plants, PSbMV had a percentage of 73.33% found only in Tehran Province (Varamin).

## PP-1_17: Mixed viral infection in sunflower plants

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Results of studying of mixed viral infection in sunflower plants in different ecological regions of Ukraine are presented in this work. The sensetivity to different species of sunflower of the Ukrainian and foreign origin to the most widespread pathogens of viral ethiology was determined. Tested species and lines are infected by the isolates of *Tobacco mosaic virus* (TMV), *Alfaalfa mosaic virus, Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus*. These conclusions were proved by the methods of electron microscopy, indirect enzyme-linked immuno assay, method of plants indicators, immunoblotting and analysis of viral proteins using electrophoresis. Morphological, structural and antigennic properties of viruses are studied. It was proved that all these viruses are the members of appropriate taxonomic groups. Seed infection was confirmed for TMV and CMV. The infection of sunflower by microscopic fungus *Sclerotinia sclerotiorum* on the background of mixed infection was observed. The system of decreasing of virus multiplication using the treatment of the seed and plants in the process of their ontogenesis by the biological stimulators and chemical compounds of different origin is developed. Some of these viruses were selected for the carrying out of different model experiments.

## PP-1_18: The new highly pathogenic strain of *Potato virus X*

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*Potato virus X* (PVX) is a rod-shaped filamentous virus of world-wide distribution in all potato-producing countries. PVX occurs in various cultivated plants, such as potato, tomato and tobacco. We have isolated and described a new strain of PVX, named PVX-Oka., We compared the symptoms on different plants infected by PVX-Oka, PVX-UK3 and PVX-Russian strains. Incubation period of PVX-Oka in *Nicotiana tobaccum* and *Chenopodium amaranticulum* is short and severe symptoms appeared rapidly. Unlike PVX-Russian and PVX-UK3 strains, PVX-Oka systemically infected *Lycopersicon esculentum*. The symptoms on *Datura Stromonium* leaves infected by PVX-Oka were more strongly marked. The new strain of PVX-Oka was studied by electron microscopy. Its structure was identical to the Russian and UK3 strains of PVX. RNA and viral protein was characterized. In vitro translation of genomic RNA yield one predominant polypeptide 165 kDa similar to that of Russian strain. The complete cDNA sequence corresponding to the genomic RNA of Oka strain was obtained. The sequence (6436 nucleotides) contains five open reading frames coding for polypeptide with molecular weight of 165 kDa, 25 kDa, 12 kDa, 8 kDa, and 25 kDa. Comparison of amino acid sequences shows an average homology of 97% for Russian isolate and 94% for UK3. It was revealed that the main differences in the nucleotide sequence of viral proteins between new strain and Russian strain of PVX located in the viral RNA-dependent RNA-polymerase (RdRp) gene (17 substitutions, of which 9 were significant). We suppose that these differences determine high pathogenicity of strain PVX Oka. In order to identify the site or sites of RdRp-gene responsible for differences in the symptoms caused by PVX Oka various chimeric DNA clones were constructed. We have designed a set of viral vectors on the basis of cDNA copy of Russian PVX strain in which different regions of polymerase gene has been changed on the corresponding sequences of PVX Oka. Thus, we are going to localize the region of RdRp Oka which could be responsible for such dramatic changes in pathogenicity.

## PP-1_19: Prevalence of potato viruses in the Punjab state, India

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Commercially potatoes are propagated primarily through the tubers and most of the associated viruses are transmitted by these seed tubers. More than 40 viruses have been reported to be associated with potato. Continuous use of virus infected seed tubers results in severe quantitative loss to potato crop. Punjab, being a major seed supplier to the other potato growing states of the country, plays an important role in disseminating these viruses. The potato growing areas of Punjab state i.e. all the 17 districts were surveyed periodically to record the symptoms, variability, incidence and distribution of viruses and were diagnosed serologically during September 2006 to April 2007. Leaf roll, mild mosaic and severe mosaic type of symptoms were observed in the field. The incidence varies from 0-100%. Maximum disease severity of 3.66% was recorded in district Hoshiarpur and minimum 0.77% was recorded in district Ropar. Serological assay revealed that the PLRV was associated with leaf roll type of symptoms, where as PVX and PVY with mild and severe mosaic. However, PVA and PVS were also observed in some samples showing severe mosaic type of symptoms. Distribution pattern showed PVX and PVY were present in all the potato growing districts of Punjab, PVA was present in districts Faridkot and Ludhiana, where as PVS was present in Faridkot, Ferozepur, Jalandhar, Mansa and Muktsar districts. Mixed infection of PVA and PVX were recorded in districts Faridkot and Ludhiana. PLRV was mainly present in Ferozepur, Faridkot and Ropar district. From the above studies, it is concluded that 5 viruses (PLRV, PVX, PVY, PVA and PVS) are associated with the potato crop in the state.

## PP-1_20: Variability of *Sri Lankan cassava mosaic virus* (SLCMV) in Tamil Nadu, India

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Cassava (*Manihot esculenta* Crantz.) is the major tuber crop in tropical and sub-tropical Africa, Asia and Latin America where it is the basic staple crop for 500 million people and one of the most reliable and cheapest sources of food. The major constraint in cassava production in Africa and India is cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses. The disease is increasing in great magnitude as the causal agent is continuously evolving its genome by recombination, pseudorecombination and mutations, resulting in more number of strains and species. Hence, it is important to know the variability of the virus and its prevalence in order to manage the disease efficiently. For this study, CMD infected cassava samples were collected from forty eight different places of Tamil Nadu. DNA was extracted from all these samples and the presence of SLCMV was identified in all the samples through Multiplex PCR. Replicase associated gene (AC1), Transcriptional activator gene (AC2), Replication enhancer gene (AC3) and unknown gene (AC4) were partially sequenced from the *Cassava mosaic virus* infected samples collected from ten different places *viz.,* Namakkal, Alwarkuruchi, Gopichettypalayam, Kanyakumari, NagarKovil, Sathiyamangalam, Dharmapuri, Erode, Salem and Coimbatore. Nucleotide sequence of all the above mentioned genes of the ten isolates were compared with each other as well as the sequence of SLCMV collected from GenBank. Cluster dendrogram was constructed individually for AC1, AC2, AC3 and AC4 gene based upon the nucleotide sequence of all the four genes of above mentioned isolates and some of the isolates submitted in GenBank. In cluster dendrogram analysis of AC1 gene, Combatore, Namakkal, Alwarkuruchi and Gopichettypalayam isolates were grouped under first cluster; Salem, Kanyakumari, Sathiyamangalam and Nagarcoil isolates were grouped under second cluster; Dharmapuri and Erode isolates were grouped under third cluster. In cluster dendrogram analysis of AC2 gene, Salem isolate alone was grouped under first cluster; Gopichettypalayam, Combatore, Namakkal and Alwarkuruchi isolates were grouped under second cluster; Dharmapuri, Nagarcoil, Kanyakumari, Erode and Sathiyamangalam isolates AC2 were grouped under third cluster. In cluster dendrogram analysis of AC3 gene, Nagarcoil isolate alone was grouped under first cluster; Salem, Gopichettypalayam, Namakkal and Sathiyamangalam isolates were grouped under second cluster; Kanyakumari, Sathiyamangalam, Coimbatore, Dharmapuri and Erode isolates were grouped under third cluster. In cluster dendrogram analysis of AC4 gene, Sathiyamangalam isolate alone was grouped under first cluster; Namakkal and Gopichettypalayam isolates were grouped under second cluster; Coimbatore, Salem, Alwarkuruchi, Kanyakumari, Erode, Nagarcoil and Dharmapuri isolates were grouped under third cluster.

## PP-1_21: Occurrence and characterization of *Bean common mosaic virus* on vanilla (*Vanilla planifolia* Andrews) in India

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Vanilla (*Vanilla planifolia* Andrews) is an important spice crop cultivated for flavoring. Madagascar, Indonesia, China, Reunion, French Polynesia and India are the countries mainly involved in vanilla cultivation. Viral infections pose a major threat to vanilla cultivation leading to considerable yield loss. *Cucumber mosaic virus* and *Cymbidium mosaic virus* are known to infect vanilla in India. In the present study, *Bean common mosaic virus* (BCMV; genus *Potyvirus,* family *Potyviridae*), found associated with vanilla in India, was characterized based on biological and coat protein (CP) nucleotide sequence properties. Mechanical inoculation tests on seven different hosts belonging to Chenopodiaceae, Fabaceae and Solanaceae showed that the virus was found to infect *Chenopodium amaranticolor, Nicotiana benthamiana* and *Vigna unguiculata*. The virus caused necrotic local lesions on *C. amaranticolor;* mosaic and leaf distortion on *N. benthamiana* and mosaic, reduction and distortion of leaf on *V. unguiculata* cv. C-52, C-152 and Kanakamani. *N. benthamiana* and *V. unguiculata* were found to be good propagative hosts for virus purification. Electron micrographs of partially purified preparations from *C. amaranticolor* showed flexuous filamentous particles of about 800 x 12nm. From a total of 25 infected vanilla samples, BCMV CP region specific primers amplified a ~800bp region of the virus from two vanilla samples in reverse transcription-polymersase chain reaction. The resulting RT-PCR product was cloned and sequenced. Sequenced region contained a single open reading frame of 809 nucleotides potentially coding for 269 amino acids. Sequence analyses showed greatest identity with BCMV indicating that the virus infecting vanilla in India is a strain of BCMV. This is the first report of BCMV infecting vanilla in India.

## PP-1_22: Emerging diseases caused by begomoviruses in sub-temperate regions in India

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Begomovirus-caused diseases are a major constraint to the production of important crops such as tomato, chili, bean, cassava and cotton, mainly in tropical and sub-tropical parts of the world. But with changing agricultural practices and ecological conditions as well as global trade in agricultural products and introduction of whitefly vector, *Bemisia tabaci*, these viruses have spread into more temperate regions like Himachal Pradesh. Recombination in begomoviruses is very frequent which increases the probability of new virus disease emergence in previously unaffected crops and regions. During surveys in various zones of Himachal Pradesh, many economically important crops such as tomato, chili, potato and papaya were found to show yellow leaf curl and mosaic type symptoms typical of begomovirus infection. Samples were collected screened with slot-blot hybridization and PCR diagnostics. Amplifications of expected size (1.2-1.4kb) were obtained. PCR amplified fragments were cloned and sequences obtained showed similarity with recently described begomoviruses reported from tomato, cotton and cucurbits. Although the genes were partial, the level of similarity from tomato isolate was less than 90% indicating that the characterized virus is a new strain belonging to family *Geminiviridae.* Full viral DNA was amplified by rolling circle mechanism using bacteriophage Φ29 DNA polymerase. Amplified products were subjected to a series of digestions with different restriction endonucleases to select an enzyme that has a unique site in the circular viral DNA. Full DNA-A from potato isolate was cloned in pUC19 vector by this method and sequenced. Sequence obtained showed high similarity with *Tomato yellow leaf curl New Delhi virus*, potato isolate. These studies show that begomoviruses have invaded into previously unaffected regions of India and are emerging as a new threat to sustainable agriculture in Himachal Pradesh.

## PP-1_23: Spread of virus diseases of fruit crops in Ukraine

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In 2004-2006 rootstock's and fruit nurseries and productive orchard were inspected by Laboratory of Virology of Institute of Horticulture in 20 farms of 17 regions of Ukraine. Following viruses were tested by DAS-ELISE: *Apple mosaic virus* (ApMV), *Apple chlorotic leafspot virus* (ACLSV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), *Prunus necrotic ring spot virus* (PNRV), *Prunus dwarf virus* (PDV), *Plum pox virus* (PPV), *Cherry leaf roll virus* (CLRV). The diagnostics was carried out to determine the quantitative and qualitative composition of the virus infection and to reveal virus-free patterns for creation clones and stocks bank in Ukraine. Of 6131 samples tested, 18.6% samples were infected by viruses. Substantial differences in the level and species of viral infection depending on crop, variety and age of orchards or nurseries were observed. The ACLSV was the most widespread in all types of apple orchards (37.4%). Among 78 apple varieties inspected the highest level of viral infection was recognized in the varieties which have been cultivated in Ukraine during long time (70.1%). In the local varieties level of viral infection was considerably low (54.4%). The most dangerous fact is the recognition of high infection level in experimental (collection and breeding) orchards of apple (38.2%). In this type of orchards, 35.3% samples infected by ACLSV, 20% by ASPV, 1.2% by ASGV and 5.8% by ApMV. According to our investigation infection level of the rootstocks depends on age. In the nurseries, which were used during 9-11 years, 25-100% of the tested samples were virus infected. In the young nurseries which were planted using certificated material imported from Europe were free from virus infections. At the same time, in the nursery of Department of Virology, which was planted in 1987 were also free of virus infections. This study showed evidence of wide spreading of ASPV in all types of pear trees orchards. This virus was detected in 36.7% of pear cultivars samples and in 8.3% of pear rootstocks samples. Average level of virus infection of the stone fruit crops was 22.6%. Among the samples tested 1.3% was infected by PPV, 2.1% by PDV, 13.6% by PNRV and 11.4% by CLRV. ACLSV and ApMV in stone fruit crops were not detected. The most significant level of virus infection was fixed in varieties of cherry (34.6%), sweet cherry (33.2%), plum (24.3%), myrobalan plum (18.5%). Varieties of apricot and peach have 8.9% and 34% samples infected, respectively. Results of this investigation revealed wide spread virus infections in all types of the orchards in Ukraine. The main direction for virus diseases control requires transfer of the nursery practice on the virus-free basis and introduction of the State System of the Planting Stock Certification. According to the testing results pure samples were selected for the creation of the virus-free certified propagation stock.

## PP-1_24: The role of olfaction in differential settling by the green peach aphid on *Potato leaf roll virus*-infected and non-infected potato plants

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We examined the role of olfaction in differential settling by the green peach aphid (GPA) onto *Potato leaf roll virus* (PLRV)-infected and non-infected potato plants. The antennae of apterous GPA were surgically removed and settling patterns of antennectomized GPA and untreated controls with intact antennae were compared in a dual-choice bioassay. In each of 11 replications, 30 aphids were released into a container from which they could climb to a platform in contact with two leaflets: one from a PLRV-infected plant and one from a non-infected plant. The location of aphids was recorded hourly for the first three hours and after 12 hours. The antennectomized GPA settled equally on PLRV-infected and non-infected plants, whereas GPA with intact antennae settled more on PLRV-infected plants. Emigration bioassays were conducted using synthetic volatile organic compound (VOCs) blends made to mimic headspace from PLRV-infected and non-infected potato plants. The rate of emigration of antennectomized aphids did not differ from the vicinity of paper strips treated with the two blends, but intact aphids were arrested by the blend mimicking PLRV-infected plants. Emigration rates from non-treated paper strips (no VOCs) did not differ between antennectomized and control aphids, indicating that surgery did not affect aphid mobility. Our results show that the GPA is guided by olfactory cues in discriminating between PLRV-infected and non-infected plants and suggest that gustatory and tactile cues are not involved.

## PP-1_25: The contribution of using healthy looking planting material in the control of sweet potato virus disease under the subsistence sweet potato production in Uganda

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In Uganda, sweet potato is the second most important food crop after cassava, but its production is greatly constrained by *Sweet potato virus disease* (SPVD) that causes yield reduction of over 58%. SPVD is caused by co-infections of *Sweet potato chlorotic stunt crinivirus* (SPCSV) with *Sweet potato feathery mottle potyvirus* (SPFMV) and/or *Sweet potato mild mottle ipomovirus* (SPMMV) that result in synergistic interactions manifesting as elevated disease symptoms, and titres of SPFMV and SPMMV. *Sweet potato chlorotic fleck carlavirus* (SPCFV) is the fourth prevalent virus and is of less importance in the country. Farmers avoid the systemic perpetuation of viral diseases through selection of healthy looking vines as the source of their planting material. The efficacy and contribution of this practice to control SPVD had yet not been evaluated especially under the subsistence manner in which farmers grow sweet potatoes. NCM-ELISA was carried out for the four viruses in healthy plants sampled from farmers' fields in the major sweet potato growing districts of Uganda, and for plants from a re-infection field experiment at Makerere University Agricultural Research Institute, Kabanyolo in central Uganda. NCM-ELISA results were confirmed by plant infectivity assays and RT-PCR using virus-specific primers. Over 87% of the healthy looking plants sampled from farmers fields tested positive for atleast one of the four viruses. SPCSV alone recorded the highest incidence (71%) followed by the co-infection of SPCSV+SPFMV (8%) and other viruses and virus combinations recorded an incidence of 7%. Re-infection data, at the end the experimental period of 6 months, showed that SPMMV and SPCSV hardly spread into sweetpotato fields planted with virus indexed vines. Only 3 and 12 plants out of 368 tested positive for SPMMV and SPCSV, respectively. The aphid-borne SPFMV spread rapidly (>72%) into the experimental fields field probably from some alternate neighbouring hosts. These results suggest SPFMV to be the limiting virus in the development of SPVD in the farmers' fields. Appreciable within field spread was observed only when a source of inoculum (4%) of the respective virus was included in the experiment; where over 80% of the plants got infected with SPFMV or whitefly-borne SPCSV. The results show that selection of healthy looking vines as planting material may not eliminate the inoculum, but probably maintains it a low levels since the mixed infections that would lead to elevated titres are be avoided at planting. Farmer selected vines may act as a reservoir for the virus(es) especially SPCSV (most frequently in healthy looking vines) such that when a plant gets infected with a second virus (e.g. SPFMV) SPVD develops. Probably, SPCSV and SPMMV transmission via planting materials is more important than for SPFMV that is more rapidly spread by vectors. Therefore, virus elimination efforts could be useful means of managing yield losses associated with SPCSV and SPMMV since their rates of re-infection could be low. Thus, without a deliberate virus eradication system, farmers may not obtain virus free planting material from selecting symptomless vines for the next crop.

## PP-1_26: Major viral diseases incidence on important vegetable crops in Hyderabad, India

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India is the second largest producer of vegetables after China. The area existing under vegetable cultivation is 6.5 million ha (6.2 to 7.0 % of the total cropping area) with the production of 108 million t per year. Although, India contributes 14.4% of the world vegetable production but the average productivity of vegetables is 13.9 t/ha. There are several factors that limit the vegetables productivity, mainly diseases and insect pests which inflict losses to the tune of 40%. The extent of crop losses in vegetables varies with the plant type, location, pathogen/vector population, disease potential of the pathogen involved and the cropping season. Therefore, constant observations were taken for important disease incidence on major vegetable crops in Hyderabad surrounding farmers' field and experimental field of AVRDC-Regional Center for South Asia during 2006 and 2007. The diseases incidence and severity in vegetable crops varied with respect to the crop and locations. The maximum incidence of *Cucumber mosaic virus* (CMV), leaf curl disease and *Peanut bud necrosis virus* (PBNV) in chilli crop was up to 85, 80 and 50 % in the month of September, May and January, respectively. However, the highest, incidence (80%) of *Yellow vein mosaic virus* (YVMV) in okra was recorded in the month of May and at the same time leaf enation disease incidence (up to 10%) was recorded only in the Secunderabad farmers field. The maximum incidence of *Tomato leaf curl virus* (ToLCV) (80%) and PBNV (100%) were recorded at Secunderabad field in the month of May and September, respectively, whereas, the highest CMV incidence (up to 80%) in cucumber was observed in the months of August and September. The correlation of disease incidence was positive with the weather data (relative humidity, temperature at ICRISAT) with respect to insect vectors population. The study clearly indicates the importance of weather data, disease vector relationship and the major disease of these locations for conducting future research on forecasting and management aspects.

## PP-1_27: Association of a *Begomovirus* complex with yellow mosaic disease of jute in India

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Jute (*Corchorus capsularis* L. and *Corchorus olitorius* L.), one of the most important cash crops of India being cultivated for its bast fibre. The crop suffers from many diseases amongst which yellow mosaic disease is of recent importance. The disease is characterized by appearance of small yellow flakes on leaf lamina in the initial stage which gradually increases, intermingled with green patches and gave a yellow mosaic appearance. The disease is found to be transmitted through whiteflies (*Bemisia tabaci*). Survey for the last three years indicated rapid increase in the incidence of this disease from 20% in 2004 to 40% in 2007 and thus necessitates immediate attention. It has also been observed that the disease is mainly restricted to *capsularis* species. Looking into the severity of the disease attempt was made to identify the virus and to study the biochemical alterations caused by the pathogen within the host plants. Since such type of symptoms are associated with *Begomovirus* an attempt was made to confirm such association with the diseased samples. Accordingly, *Begomovirus* specific different primers for DNA A, DNA B and DNA beta were employed which amplified a 1.2 kb fragment of DNA A, 2.0 kb fragment of DNA B and 1.3 kb fragment of DNA beta. Cloning and sequencing of 1.2 kb fragment revealed that it consisted of 1263 nucleotide (EU047706) and shared highest similarity (91.2%) with *Corchorus golden mosaic Vietnam virus*. Phylogenetic analysis with other begomoviruses revealed that this particular isolate appeared as a distinct one. Cloning and sequencing of other fragments are in progress. Studies on biochemical alterations in plants infected with this disease indicated higher amount of peroxidase, esterase and catalase in leaves of infected plants than healthy ones.

## PP-1_28: Biological differentiation of furoviruses in cereals

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The two furoviruses *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV) represent an increasing threat for the commercial production of several cereal crops in Germany and other European countries. Both viruses are known for their considerable genetic diversity. In the frame of a research programme on soil-borne cereal viruses 15 German isolates of SBCMV and 2 isolates of SBWMV (Heddesheim/Germany and ATCC-PV-65 Nebraska/USA) were analysed for their biological properties on winter forms of wheat, triticale, rye and barley and on various indicator plants like *Gomphrena globosa, Nicotiana benthamiana* and several *Chenopodium* spp. Infection tests were performed both in naturally infested fields and in a climate chamber at 17°C with mechanical inoculation of leaves. Plants were analysed for virus content by DAS-ELISA, IC-RT-PCR and Immunosorbent Electron Microscopy (ISEM) using specific monoclonal antibodies for SBCMV and SBWMV, respectively (Rabenstein et al., 2005). After natural transmission by the plasmodiophorid vector *Polymyxa graminis* SBCMV was detected in all species but not in winter barley while SBWMV was able to infect this species too. Therefore, barley is a suitable host to discriminate between the two related viruses in infested soils. In contrast, after mechanical inoculation of leaves SBCMV was also detected in barley plants, but always in very low amounts. None of the two viruses was able to infect plants of *Gomphrena globosa*. Both induced almost identical symptoms in different *Chenopodium* spp. but they could be discriminated on *Nicotiana benthamiana*. While inoculated plants became fully systemically infected by all SBCMV isolates showing yellow leaf spots after 3 weeks and complete dying of plants after 6 weeks infections with the 2 SBWMV isolates always remained local. Hence, *Nicotiana benthamiana* can be used to biologically discriminate between these two furoviruses.

## PP-1_29: Occurrence of furoviruses in winter barley

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*Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV) are transmitted by *Polymyxa graminis* Ledingham and known as important pathogens causing severe yield losses in commercial production of winter wheat, winter rye and winter triticale in several European countries and Northern America. In contrast to these species barley seems to be not seriously affected although the first report on the occurrence of SBWMV in this crop was given by McKinney already in 1948. In Germany this virus was detected for the first time in a field grown plant of winter barley in 2005 using DAS-ELISA, IC-RT-PCR and the ISEM technique. To fulfil Koch`s postulates young seedlings of wheat, rye, triticale and barley were inoculated mechanically with sap of infected leaves in a climate chamber. As a result plants of all species became infected. Subsequently, they served as specific inocula to successfully retransmit the virus to winter barley plants. These data indicate that since SBWMV is able to attack barley under field conditions the virus constitutes a potential novel threat to barley production. In a second approach all 4 cereal species were grown in fields naturally infested with SBCMV at 4 different locations in Germany over a period of 3 years. While the wheat, rye and triticale material easily became infected the presence of SBCMV in barley plants was never detected. In contrast to the natural conditions with fungus-mediated virus transfer, barley seedling grown in a climate chamber could be successfully infected by mechanical inoculation, but in this case the concentration of the pathogen remained always very low. In a next step 50 varieties and accessions of winter barley were selected, that represented all 16 currently known recessive genes conferring resistance against the two yellow mosaic causing viruses (*Barley mild mosaic virus*, *Barley yellow mosaic viruses*). This material was grown in the SBWMV-infested field in Heddesheim/Germany and assessed for resistance behaviour against the furovirus. As the experiment revealed clear differences in the susceptibility level first putative donors of resistance against SBWMV in barley could be selected among these genotypes.

## PP-1_30: *Plum pox virus* epidemiology in nursery blocks of *Prunus* under high inoculum pressure

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Sharka disease, produced by *Plum pox virus* (PPV), continues to be detected in many countries and is currently spreading in new areas, causing important economic losses on fruiting, ornamental and wild *Prunus* species. To try to reduce the natural spread of PPV infection an improved sanitary control of propagation material and of nursery plants is needed, as well as the development of cultivation strategies based on PPV epidemiological data. However, although there is now significant knowledge on the biological behaviour of PPV in orchards, there is currently a great lack of information about the epidemiological behaviour of PPV in prunus rootstocks grown in blocks in open field before grafting with PPV-tested cultivars. Several parameters governing the epidemiology of PPV were studied in an experimental nursery in Valencia (Spain), during 2006-2007, established in an area with very high prevalence of the disease (about 80% of infected adult trees). The susceptibility to PPV-D of the most widely used prunus rootstocks, the identification of the aphid species and period of activity visiting nursery blocks, the number of PPV-viruliferous aphids present in the field visiting a single plant and the efficiency of oil treatments as a control strategy to reduce the spread of PPV, were evaluated in open field. The susceptibility to PPV of the prunus rootstocks was assessed by DASI- ELISA (5B- IVIA) and real-time RT-PCR. The infection rate detected in Spring 2007 was: Mariana GF8-1 (*P. cerasifera x P. munsoniana*) (63.52%), pollizo de Murcia Adesoto 101 (*P. insisitia*) (43.75%), Nemaguard (*P. persica x P. davidiana*) (26.69%) and Myrobolan 29C (*P. cerasifera*) (18.52%). PPV was not detected in blocks of Garnem GxN 15 (*P. dulcis* Garfi x Nemared (Nemaguard x *P. persica*), Cadaman (*P. persica* x *P. davidiana*) nor in GF677 (*P. dulcis* x *P. persica*) rootstocks. To identify the aphid species associated to the nursery blocks and to determine their population dynamics and their potential as virus vectors, the sticky shoot method and yellow water-pan traps placed on the orchards were used. After their identification, caught aphid species were preserved in alcohol and analysed by real-time RT-PCR based methods to estimate the percentage of aphids carrying PPV-targets. The main aphid species landing on the nursery blocks both years were *Aphis spiraecola* (56%) and *A. gossypii* (4%). Peak populations of aphid species occurred in May. The proportion of PPV-viruliferous aphids of the main aphid species were approximately 30%. The efficiency of oil treatments in the PPV spread was evaluated in experimental blocks of Mariana GF8-1 and Nemaguard rootstocks (both very sensitive to natural PPV infection). Mineral oil (Sunspray Ultrafine 1%) treatments were performed every 10-12 days in these blocks following a statistical design and PPV incidence was assessed in treated and non-treated blocks. The PPV infection in Mariana (63.52%) resulted significantly different to the Nemaguard (26.69%) infection. PPV infection was reduced in both cases when oil treatment was applied being this difference significant only for Mariana rootstocks (48.81 %). The combination of oil treatments, grafting with PPV-free cultivars and the selection of the less PPV-susceptible rootstocks could contribute to reduce the trade of infected plants from nurseries.

## PP-1_31: Influence of an alternate weed host, *Solanum sarrachoides* (sendtner) on the epidemiology of *Potato leaffroll virus* in potato ecosystems of Idaho

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Idaho is the largest potato producing state in the United States. However, potato production is currently jeopardized by an array of viruses. *Potato leafroll virus* (PLRV) is one of the important viruses that severely affect potato yield and quality. *Potato leafroll virus* is a *Luteovirus*, and is persistently transmitted by two important colonizing aphids, the green peach aphid *Myzus persicae* (Sulzer), and the potato aphid *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae). Hairy nightshade, *Solanum sarrachoides* (Sendtner) is a solanaceous weed omnipresent in Idaho's potato ecosystems. This weed directly competes with the crop for water and nutrients, besides it also serves as a reservoir for vectors mentioned above and PLRV. Hairy nightshade and potato are in the same genus '*Solanum*', this taxonomic proximity does not permit selective herbicidal control and thus complicates vector and virus management. Field observations indicated that both *M. persicae* and *M. euphorbiae* preferentially settled on hairy nightshade than on potato. Subsequent laboratory experiments revealed an enhanced performance of both *M. persicae* and *M. euphorbiae* on hairy nightshade than on potato. Their performance further enhanced with PLRV-infection. Subsequent transmission experiments indicated that *M. persicae* transmitted PLRV more efficiently from hairy nightshade to potato than from potato to potato. These observations lead to the hypothesis that hairy nightshade under ideal field conditions could potentially serve as spatially separated inoculum sources and affect the vector population dynamics and subsequently PLRV-spread. Two field trials across two field seasons 2004 and 2005 were conducted in Kimberly, Idaho, USA to assess the role of hairy nightshades in vector population dynamics and PLRV-spread. In the first trial non-infected hairy nightshades were planted in the middle of a potato field and vector population dynamics and PLRV-spread was monitored at regular intervals. In the second trial PLRV-infected hairy nightshades were planted in the middle of a potato field, as in the previous case vector population dynamics, and spatial and temporal disease spread was monitored. The mere presence of hairy nightshade in trial 1 clearly increased vector populations and favored PLRV-spread. In the second trial too, a very similar phenomenon was observed with vector populations. Spatial and temporal disease monitoring clearly indicated an increased spatial and temporal virus spread in plots with PLRV-infected hairy nightshade than in plots with PLRV-infected potato. These results clearly demonstrate that hairy nightshade can affect the PLRV epidemiology by acting as a vector reservoir as well as a viral inoculum source. Hairy nightshade is also a host of several other important potato viruses including *Potato virus Y* and is also a prolific seed producer, thereby increasing the pathosystem complexity. Understanding the role hairy nightshade in the potato-PLRV pathosystem, has increased the knowledge of the pathosystem and currently management plans are being devised with special emphasis to hairy nightshade management.

## PP-1_32; Occurrence of some viruses and viroids on stone fruits in the Czech Republic

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Stone fruits can be infected by a lot of viruses. Besides well known, economically very important and widespread viruses like *Plum pox virus*, *Prunus necrotic ringspot virus* and others occurrence of probably less harmful and sometimes symptomless viruses and viroids has been studied in the Czech Republic during last several years in the frame of a grant aiming to adopt diagnostic methods for the certification system of planting material of stone fruits. Samples were taken from both commercial orchards and germplasm of apricots, peaches, plums, and cherries. The number of samples varied according to the species and virus and/or viroid tested but usually several hundreds of samples were collected. They were analyzed by RT-PCR adopted in our laboratory for the detection of *Apricot latent virus* (ALV), *Cherry virus A* (CVA), *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd). Appropriate positive and negative controls were used. PLMVd was proved to be the most widespread from these pathogens. In some cases it was found in more than 50 % of peach trees but no other species was infected by this viroid. HSVd seems to be present in some cases of peach trees and sometimes mixed infection with PLMVd was found. Both single and mixed infections by the viroids were symptomless under our conditions. Recently, some cherry trees have also been positively tested for the presence of CVA but the results have yet to be confirmed. Till now, the ALV has not been found in apricots. High percentage of peaches infected by PLMVd and/or HSVd shows the need to establish certification system of planting material with all its implications as soon as possible to prevent further spreading of these pathogens.

## PP-1_33: Sweet potato leaf curl disease - a new emerging virus problem of sweet potato in India

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Sweet potato (*Ipomoea batatas* (L) Lam.) ranks seventh in food production worldwide and third most important root & tuber crop after potato and cassava. It is an important tuber crop grown in different parts of India, which are used as subsidiary food and also an industrial raw material for production of starch. Sweet potato is affected by several viral diseases which reduce the yield and quality of the storage roots. At present, there are more than 15 viruses known to infect this crop. In India, occurrence of *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV) and *Sweet potato chlrotic fleck virus* (SPCFV) were reported earlier. Recently, we observed that some of the sweet potato lines showed leaf curl symptoms. The present investigation aims at symptomatology, identification and characterization of the causative virus. Symptomatological observations showed the curling of the leaf upward, folding of leaf lamina in all sides which produce cupping of the leaves. Graft transmission of *Ipomoea setosa* produced leaf curl and vein clearing symptoms. Total DNA was isolated from the infected plants and subjected to PCR using gemini group specific primer which has yielded positive amplification of 530 bp. PCR analysis of infected lines with different sets of *Sweet potato leaf curl virus* (SPLCV) specific primers showed amplification of desired product size of 514, 912, 1148 and 2400 bp for primer pairs of PW1/2, G1/2, G3/4 and PW 3 / 4 respectively. Analysis of the sequence of PCR products showed close relationship with published SPLCV sequences. Comparison of the above findings with available published information on SPLCV confirms that the disease in the present study is caused by *Sweet potato leaf curl virus, begomovirus, Geminiviridae*. This is the first report of occurrence of SPLCV in India.

## PP-1_34: Occurrence and distribution of viruses infecting fluted pumpkin (*Telfairia occidentalis*, Hook F.) in Imo State, Nigeria

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Fluted pumpkin (*Telfairia occidentalis,* Hook F.) is a drought tolerant tropical vine that is fast assuming great importance in West, Central and East Africa because of its nutritional value and income generating potential particularly for the rural farm families. It is by far the most popular leafy vegetable for the Igbo tribe in Eastern Nigeria especially Imo State which has the highest diversity of this plant's population. Although pests like *Zonocerous variegates, Copa occidentalis* and fungi including *Phona sorghina, Fusarium oxysporium* are constraints to its production, none of them are of more economic importance than those caused by viruses. Five viruses, viz., *Cucumber mosaic virus* (CMV; genus *Cucumovirus*), *Pepper veinal mottle virus* (PVMV; genus *Potyvirus*), *Telfairia mosaic virus* (TeMV; genus *Potyvirus*), *Watermelon mottle virus*-2 (WMV-2; genus *Potyvirus*) and *Zucchini yellow mosaic virus* (ZYMV; genus *Potyvirus*) are known to infect this crop worldwide, but only TeMV had been recognized in Nigeria during earlier surveys conducted in 1987-89. Currently limited information is available on the occurrence, incidence and distribution of virus infecting *Telfairia*. This survey study was conducted in 2007 to address these parameters in Imo State, number one in telfairia producer in Nigeria. Survey was carried out in farmers' fields (size range between 0.1 to 0.5 ha) and home gardens of 75 villages in 15 Local Government Areas (LGAs) of Imo State (survey region lies between latitudes 4″45′N and 7″15′ N; longitudes 6″50′ E and 7″25′E and covers an area of 5,100 sq.km). Three leaves showing virus-like symptoms and asymptomatic ones were collected randomly from two farms in each village. They were indexed for TeMV, PVMV, and ZYMV and CMV by Protein A antibody sandwich-enzyme linked immunosorbent assay (PAS-ELISA) using respective antibodies. Further identification of viruses was made by biological indexing using representative samples for each virus to selected indicator plants (*Chenopodium amaranticolor, C. quinoa, Nicotiana benthamiana* and *Vigna unguiculata*). Results showed that of 225 samples collected, 27.5% samples tested positive for ZYMV, CMV or TeMV, but none of them tested positive for PVMV. TeMV was the most prevalent (10.2%) followed by ZYMV (9.7%) and CMV (8.4%). Also, telfairia grown in 11 of the LGAs were virus infected with Owerri North having the highest (14 out of 15 samples), while telfairia grown in Ezi-Nihitte, Mbano, Mbaitoli and Okigwe are free from these viruses. Incidences of ZYMV, TeMV and CMV were highest in Aboh Mbaise (53.3% of samples collected), Owerri North (40.0%) and Njaba (26.7%), respectively. Mixed infection with these viruses was noted in 14 samples (6.2%). This is the first report of CMV and ZYMV occurrence on telfairia, and the first report of ZYMV in Nigeria. Occurrence of CMV and ZYMV is a cause for concern because of their wide host range and high potential for rapid spread by aphid vectors or even through seed. Further studies are necessary to access the economic losses due to these virus infections and occurrence and distribution of ZYMV in Nigeria.

## PP-1_35: Incidence and distribution of virus and virus-like diseases on yams (*Dioscorea* sp.) in the Republic of Benin

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Yams are economically important staple food contributing more than 200 dietary calories per person each day for millions of people in Africa. Viral pathogens are amongst the most important of the many factors that have deleterious effects on yam tuber yield and quality. Information on virus disease incidence assists extension workers and growers in making decisions on the procurement and dissemination of healthy planting materials. A survey was conducted in 2005 to determine the incidence, severity and distribution of virus and virus-like diseases on *Dioscorea alata, D. dumetorum* and *D. rotundata* in Zone Vivrière du Sud Borgou, Zone Ouest-Atacora, Zone Cotonnière du Nord Bénin and Zone Cotonnière du Centre Bénin in the Republic of Benin. A total of 1175 yam plants were scored for presence or absence of symptoms in 45 yam fields. Symptom severity on individual plants was assessed on a scale of 1-5 based on percentage of leaves infected (1=No obvious symptoms; 2 = symptoms on 0 to 25% of leaves; 3 = symptoms on 25-50% of leaves; 4 = symptoms on 50-75% of leaves; and 5 = symptoms on 75 –100% of leave). *Dioscorea rotundata* was found in all the fields surveyed accounting for 90.4% of the total samples, followed by *D. alata* (9.3%) and *D. dumetorum* (0.3%). Virus-like symptoms were observed in all fields surveyed and the most common symptom types observed were chlorosis, mosaic, puckering, crinkling, mottling and shoe stringing. Symptom incidence ranged from 4 to 88%, with incidence in most fields between 21 to 60%. Mean symptom incidence was highest in Cotonnière du Centre Bénin (57%) and lowest in Zone Ouest-Atacora (19%), while mean symptom severity score was highest in yam fields in Zone Vivrière du Sud Borgou (mean disease score = 4.6). A total of 559 leaf samples obtained from the surveyed fields were screened for commonly occurring yam viruses, viz., *Cucumber mosaic virus* (CMV; *Cucumovirus*), *Dioscorea bacilliform virus* (DBV; *Badnavirus*), *Dioscorea mottle virus* (DMoV; unassigned Carla-like virus), *Yam mosaic virus* (YMV; *Potyvirus*) and *Yam mild mosaic virus* (YMMV), by enzyme-linked immunosorbent assay (ELISA), immuno-capture polymerase chain reaction (IC-PCR) and IC-RT-PCR. Sixty-six percent of these samples were positive to one of these viruses tested by ELISA and/or PCR, but DMoV was not detected in any of the samples. YMV was the most prevalent (40%), followed by DBV (34%), YMMV (22%) and CMV (1%). Although CMV incidence was low, this is the first record of this virus in yams in the Republic of Benin. *Dioscorea alata,* with the highest incidence of DBV (96.3%), YMMV (68.5%) and CMV (5.6%), was the most heavily infected yam species followed by *Dioscorea rotundata* which had the highest incidence of YMV (42.0%). Some samples with obvious virus-like symptoms tested negative to all the five viruses. Attempts are being made to determine association of any other virus with such symptomatic samples. This study establishes that yams are widely affected by virus diseases in the Republic of Benin. Efforts to expand yam production/yield should concentrate on breeding for virus resistance and the use of virus-free planting materials.

# Poster Session – II

## PP-2_36: Quantifying yield losses caused by plant viruses

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Plant virus pathosystems inherently present a number of challenges when scientists attempt to obtain accurate and reliable yield loss estimates. These include, but are not limited to: (i) whether to assess disease incidence (based on symptoms) versus pathogen incidence (which is based upon the results of pathogen-specific detection tools)?, (ii) the decision as to what constitutes a sampling unit (leaflet, leaf, stem, plant, plot or field)?, (iii) deciding how many sampling units should be visually assessed (symptoms) or tested to ensure the validity of the data?, (iv) when should sampling units be visually assessed (disease incidence) or tested for the presence of a virus pathogen (pathogen incidence)?, (v) how should the dependent variables (yield, yield components, and yield quality) be sampled and measured to minimize bias?, (vi) what will constitute the non-treated versus the treated controls (that may lack a truly disease/pathogen-free treatment)?, and (vii) what statistical methods/models will be applied to the data after it is collected? Time of symptom appearance/virus detection: yield reduction models are essentially stimulus-response models that allow the use of regression models and/or non parametric statistical methods to quantify disease/pathogen incidence (x)-yield/yield components (y) relationships, as affected by the interactions of host (resistance), virus strain (aggressiveness), and environment (which includes the duration in time that the host-virus interact to affect yield and the yield components. For example, time of symptom appearance (x) versus yield/plant measurements (y) provided an excellent quantitative measure of reductions in yield (kg/plant) and yield components (number of fruit/plant and fruit weight) due to *tobacco etch virus* infection in bell paper. However, in the bean pod mottle virus/soybean /bean leaf beetle pathosystem, it was the time that *bean pod mottle virus* was first detected in 30-cm soybean quadrats (by ELISA) that had the best relationship with reductions in yield and yield components (such as the number of pods per plant and seed weight in soybeans). Examples of sampling protocols, assessment protocols, and modeling approaches used to quantify disease/pathogen incidence at time (t) to quantify virus impact on yield and yield components will be presented.

## PP-2_37: Possible ways of transmission of sugar beet viruses

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Most sugar beet viruses are transmitted mechanically or with the help of some vectors. The fact of the virus transmission by seeds is not proved by any researcher. It can be assumed that these viruses are transmitted by seeds, but in what way? Detection of the viruses in the laboratory conditions using sterile soil without any vectors has led us to the assumption of the possibility of such infecting of the plant by the virus. Considering that some beet viruses can be transmitted mechanically we have presumed that the virus may get into the plant with the sugar beet plant debris during obtaining the seed grain. To check our hypothesis we have used Ukrainian pelleted and not pelleted sugar beet seeds. The seeds have been washed and stirred in buffer solution for 12 hours and solution was concentrated by the differential centrifugation. The obtained sediment was examined by electron microscopy and it contained some rod-like particles of different size which are characteristic for some viral families. This data is in agreement with the serological data obtained by us. According to the above we have assumed that the seeds with the rough surface may adsorb the plant debris containing viruses electrostatically. After pelleting the seeds the plant debris concentrated and the possibility of the virus preservation in such ‘complex’ increases. During the emergence, seedlings can be infected by the mechanical friction by the plant debris and soil particles.

## PP-2_38: Distribution of sunflower viruses in four provinces of Iran

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Sunflower (*Helianthus annuus*) is an important oilseed crop grown in many countries. Diseases present a major constraint to sunflower production and can lead to significant reductions in yield and seed quality. Some viruses damage this plant. Up to now, we don't have any report of the presence of sunflower viruses from Iran. In this survey, during 2006 growing season, to determine the distribution of potyviruses, PVY, TEV, WMV and TSV, 310 samples were collected from sunflower fields in Kerman, Golestan, Mazandaran and Tehran provinces. Infected plants included of symptoms such as: mosaic, yellowing, deformation, chlorotic and necrotic lesions and mottling on leaves and stunting of plants. Distribution of these viruses were determined by DAS-ELISA and ACP-ELISA. The percentage of potyviruses, WMV, TSV, PVY, TEV were 21%, 2%, 5%, 10% and 4%, respectively. Double infection with PVY+TEV, PVY+WMV, TEV+WMV, PVY+TSV and TEV+TSV was 1.9%, 1.29%, 0.32%, 1.29% and 0.32%, respectively. Then PVY was the most prevalent virus rather than others. This is the first report of presence of WMV and TEV on sunflower in world and first report of presence of TSV and PVY in Iran.

## PP-2_39: Occurrence of *Lettuce mosaic virus, Cucumber mosaic virus* and *Tomato spotted wilt virus* on lettuce

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*Lettuce mosaic virus* (LMV) *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV) were identified in fields of Tehran province. In this study 452 infected lettuce plants having viral infection symptoms including, mosaic, mottling, leaf distortion, stunting and lack of flower heads, were collected from the fields throughout the Tehran province. Distribution of *Lettuce mosaic virus* (LMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV) and *Arabis mosaic virus* (ArMV) determined with DAS-ELISA. LMV, CMV and TSWV were found on lettuce in this region, but not ArMV. Percentage of single infection to LMV, CMV or TSWV was 20.58%, 15.93% and 9.96%, respectively. Also 15.28% of samples were co-infected with LMV+CMV, 8.19% with LMV+TSMV and 7.74% with CMV+TSWV. 4.65% of samples were infected by all of these viruses. LMV was found in 48.69%, CMV in 43.59% and TSWV in 30.54% of samples totally. Therefore LMV is major dominant agent of lettuce mosaic disease in Tehran province. This is the first report of occurrence of TSWV on lettuce in Iran and first report of CMV and LMV in Tehran province.

## PP-2_40: Studies on monitoring and assessment of crop loss to necrosis virus disease in sunflower

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The necrosis virus on sunflower is one of the emerging disease in recent years and becoming an important constraint in the Peninsular India. The necrosis disease monitoring in Aland taluk alone in Gulbarga district in Karnataka, India, indicated that out of 23,000 ha area planted, 12,142 ha (52.79%) area suffered severe crop loss due to necrosis virus at early growth stage (45 days) during 2002-03. Further, the survey during 2003-04 and 2004-05 in three districts of Gulbarga, Bidar and Raichur indicated the mean disease incidence of 19.81% with disease ranging from 0.0–100%. The results on crop loss assessment to necrosis disease indicated significant loss both at vegetative and grain yield levels. The reduction in plant height (58.99%), number of leaves (38%), leaf area (63%), head diameter (64%), number of seed/head (91.7%), seed yield (96.7%), 100 seed weight (59.6%) and oil content (50.9%) was recorded respectively in comparison to healthy plants. The assessment of crop loss in fifteen commercial hybrids under cultivation in farmers field, it was observed that the mean grain yield loss to necrosis disease was 33.9% and the loss varied from 25 to 52%. The maximum loss in yield up to 52% was observed in cultivar Pioneer 65A-24. An attempt is in progress to identify resistance sources and to develop sustainable management practices to reduce the crop/revenue loss to sunflower necrosis disease

## PP-2_41: Shoot bug, *Peregrinus maidis* (Ashmead), a vector of sorghum stripe disease: trend in Karnataka during post rainy season on sorghum

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Sorghum shoot bug, *Peregrinus maidis* (Ashmead) previously considered to be of minor importance, but now with the introduction of new sorghum genotypes of different maturity has become a serious pest. Both macropterous and brachypterous nymphs and adults suck the sap from the leaves by congregation in the plant whorl and inner sides of the leaf sheath. Severe attack of shoot bug results in leaf chlorosis, stunted growth, shriveled and chaffy grains. The top leaves start drying first, but leaf death gradually extends to older leaves and some times, death of the whole plant occurs. Severe infestation at boot leaf stage results in twisting of top leaves thus preventing the emergence of panicles. Further, the honey dew excreted by nymphs and adults favours the growth of sooty mould fungus (*Capnodium* sp.) which inhibits the photosynthetic activity. It was also reported as a vector of sorghum stripe disease (SStD). Since the disease incidence has been on increasing trend year by year in the last one decade, a roving survey was carried out for three years during post rainy season from 2004-05 to 2006-07 in Bijapur district covering five talukas to assess its impact. In each taluka ten fields were visited randomly at around 45 days after emergence of the crop. The shoot bug population per plant was recorded. The plants showing yellowing of leaves and stunted growth symptoms were recorded. At about 60 and 75 days after emergence of the crop, the same fields were visited for recording girdling of topmost leaves without panicle development and poor panicle exertion, respectively. Similarly, the above observations were also recorded on the research farm on three varieties grown on larger area. The population of shoot bug varied from 20.51 to 26.34 per plant in different talukas of the district on farmer's fields mostly on M 35-1 variety. Where as, on research farm comparatively higher population of 36.34, 28.67 and 29.13 per plant was observed on M 35-1, DSV 4 and DSV 5 varieties, respectively. The plants showing yellowing and stunted growth varied from 6.97 to 10.61 per cent on farmer's fields over the district. On research farm, it varied from 4.46 to 14.32 per cent on three ruling varieties. The plants showing girdling of top most leaves and poor panicle exertion ranged from 2.91 to 5.54 and 1.74 to 3.56 per cent, respectively on farmer's fields. On research farm, the plants showing girdling of top most leaves and poor panicle exertion ranged from 5.88 to 7.26 and 3.48 to 4.84 per cent, respectively and were little higher compared to farmer's fields. The overall stripe virus incidence ranged from was 12.66 to 18.67 per cent over the district on farmer's field mostly on M 35-1 while on research farm, it was 26.42, 19.52 and 13.82 per cent on M 35-1, DSV 4 and DSV 5 varieties, respectively. The higher incidence of the disease was observed towards border areas of the fields as compared to the interior ones. This was due to the presence of alternate hosts like grasses growing on the bunds.

## PP-2_42: Loss estimation due to shoot bug, *Peregrinus maidis* (Ashmead) in *rabi* sorghum under field conditions

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Field trial was conducted during post rainy season of 2004-05 to estimate loss due to sorghum shoot bug, *Peregrinus maidis* (Ashmead), a vector of sorghum stripe disease (SStD) with five dates of sowing taken up at weekly intervals commencing from fourth week of September to fourth week of October. The trial was laid out in factorial randomized block design having protected and unprotected plots with three replications, using M 35-1 variety in a plot size of 3.6 x 4.5m. The crop was raised with a spacing of 60 x 15 cm by following all recommended package of practices except the plant protection schedule. In the case of protected plots the shoot bug was kept under check by spraying endosulfan 35 EC @ 0.07 per cent twice at 25 and 40 days after emergence. While in the unprotected plots, it was allowed for natural infestation of shoot bugs. The other pests were hand-picked and destroyed. The incidence of shoot bug was regularly noted both in protected and un-protected plots at 20, 30, 40, 50 and 60 days after germination on five randomly selected plants and averaged to express as mean population per five plants. The per cent crop loss in terms of grain yield, fodder yield and 1000 grain weight at different dates of sowing was calculated by using the modified Abbott's formula. Panicle emergence was also recorded at 80 days after sowing. The stripe virus disease incidence was also recorded at 70 days after sowing. Leaf sugary exudates intensity grade (1-5 scale) and plant showing the leaf sugary exudates malady were recorded on 45$^{th}$ day after sowing. The results revealed that, the over all loss of 11.16, 21.11 and 2.97% in grain yield, fodder yield and 1000 grain weight, respectively was recorded under unprotected conditions as compared to protected ones across five dates of sowings. The unprotected plot recorded significantly higher sorghum stripe virus disease incidence as compared to protected ones with 18.72 and 9.51%respectively, thus accounting for 51.26% over all increased incidences in unprotected plot over protected ones. The unprotected plot recorded significantly higher shoot bug population over protected ones with 39.87 and 3.27 shoot bugs per five plants, respectively with 92.02 per cent over all increase in population in the unprotected plot over protected ones. With delay in sowing, reduction in shoot bug population was evident. The unprotected plot was significantly inferior over protected ones with leaf sugary exudation grade of 3.70 and 1.31, respectively with 63.86 per cent overall increase in unprotected plot over protected ones across five dates of sowings. The crops sown during September IV and October I week under unprotected conditions were significantly inferior by recording 89.20 and 85.73 per cent leaf sugary malady affected plants, respectively over October II, III, IV week sown crop under unprotected and were at par with each other. As the sowing was delayed, there was decrease in the plants affected by leaf sugary malady and panicle emergence in unprotected conditions.

## PP-2_43: Serological relationship of vegetable infecting tospoviruses in India

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Tospoviruses (family: Bunyaviridae, genus: *Tospovirus*) are an emerging group of plant viruses causing devastating diseases in vegetable, legume and ornamental plants. Of the 16 characterised tospoviruses, four tospoviruses viz., *Peanut bud necrosis virus* (PBNV), *Peanut yellow spot virus* (PYSV), *Watermelon bud necrosis virus* (WBNV) and *Iris yellow spot virus* (IYSV) were reported so far from India. Monitoring studies were conducted during 2005-07 to document the prevalence of tospoviruses in vegetable crops and study their serological relationships. Natural infection of PBNV was recorded on tomato, chilli, brinjal, carrot and blackgram which produce chlorotic and or necrotic spots, rings on leaves, petiole, stem and growing bud. The natural infection of WBNV was recorded on watermelon showed mosaic mottling on leaves, necrotic streaks on petiole, stem and gowning buds. In advanced stages, the infected plants produced small to abnormal fruits with chlorotic and or necrotic rings on the surface of fruits. In onion, the natural infection of IYSV showed typical chlorotic and or necrotic spindle or oval shaped spots on scapes and flower stalks and bending of flower stalks were commonly observed. Fifteen PBNV isolates from six different crops and five WBNV isolates from watermelon and five IYSV isolates from onion were collected from six different states in India. The virus isolates were maintained on *Vigna unguiculata* or *Nicotiana benthamiana* under greenhouse conditions by mechanical sap inoculations. To study the serological relationships PBNV, WBNV and IYSV isolates were tested by DAC, DAS and TAS ELISA using polyclonal antisera (PAbs) to GBNV nucleocapsid protein (NP) and IYSV-NP and monoclonal antisera (MAbs) to WBNV-NP, *Watermelon silver mottle virus* (WSMoV) NP, non-structural proteins (NSs) and *Capsicum chlorosis virus* (CaCV) NP. All GBNV and WBNV isolates were reacted positively to PBNV-NP-PAbs and MAbs, WSMoV-NP and NSs MAbs in DAC and TAS ELISA indicated a strong serological relationship with WSMoV. WBNV isolates reacted positively with WBNV-NP-MAbs where as IYSV isolates reacted positively only with IYSV-PAbs in DAS-ELISA.

## PP-2_44: Evaluation of pigeonpea genotypes for resistance to sterility mosaic disease

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Pigeonpea (*Cajanus cajan* L.) is an important grain legume predominately grown in the Indian sub-continent. The production of pigeonpea is severely affected by sterility mosaic disease (SMD) which causes annual grain loss of worth >US$300 million. The disease is caused by Pigeonpea sterility mosaic virus (PPSMV), transmitted by an eriophyid mite (*Aceria cajani*). The objective of study was to identify the resistant genotypes of pigeonpea against sterility mosaic disease. Infector-hedge, leaf stapling and petiole grafting techniques were adopted to inoculate the different genotypes of pigeonpea. Double antibody sandwich (DAS)–ELISA was adopted to assess the PPSMV infection in promising genotypes of pigeonpea. A randomized block design with three replications was used. Eighty two genotypes of pigeonpea grown in the field were screened adopting infector – hedge and leaf stapling techniques. Thirteen genotypes were highly resistant to SMD showing no any symptoms of disease. ICP 8863 a susceptible genotype was highly affected and showed severe sterility mosaic symptom. Two sets of 22 genotypes of pigeonpea grown in pots containing field soil under glass house were separately inoculated adopting leaf stapling and petiole grafting techniques. All the genotypes except TT 701 and SM 03-17 exhibited symptom of sterility mosaic disease when inoculated adopting both leaf stapling and petiole grafting techniques. The genotype TT 701 did not show any symptom inoculated adopting both the techniques and was grouped as highly resistant to mite and PPSMV. However, genotype SM-03-17 was highly resistant when inoculated adopting leaf stapling technique but showed symptom of SMD when inoculated by petiole grafting technique. Fourteen genotypes of pigeonpea were assessed for PPSMV infection adopting DAS-ELISA. The genotypes ICP 8863, ICP 2376, ICP 11164, GAUT 011, BDN 708 and LRG 30 were highly susceptible and showed strong positive reaction. BDN 2009 and H 94-6 were moderately susceptible showed positive and JJ 65 and Purple1were susceptible showed week positive reaction. Genotypes ICP 7035, ICP 8862, TT701 and SM 03-17 were resistant and showed negative reaction.

## PP-2_45: *Pepino mosaic virus*: epidemiology, economic impact and pest risk analysis (PEPEIRA)

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PEPEIRA is a EU KP6 funded research project aimed at developing an EU-wide Pest Risk Assessment (PRA) for Pepino mosaic virus (PepMV). The project is coordinated by Plant Research International, Wageningen and involves 20 partners from 17 EU countries. It will investigate the epidemiology and economic impact of PepMV in order to allow a robust and scientifically-justified assessment of the risk posed by this pathogen to the European tomato industry. This includes a true assessment of the economic impact on tomato crops growing in Member States with different climatic and market conditions and the role of seed transmission in the spread of PepMV. The project will also address the increased risk posed by new, biologically and genetically distinct strains of PepMV that have appeared in Europe and elsewhere recently and have the potential to be far more damaging. The proposal will also address the issue of developing validated diagnostic protocols, to be published via EPPO that can be used with confidence by National Plant Protection Organisations (NPPOs) within the EU and laboratories in other countries that trade with the EU. Adoption of the new PRA will allow EU Plant Health services to develop, via Council Directive 2000/29/EC, a consensus on appropriate measures to prevent PepMV becoming significant detrimental to the EU tomato industry.

## PP-2_46: *Apple chlorotic leaf spot virus*: Incidence, epidemiology, genomic diversity and strategies for disease management

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Extensive surveys were done in the apple growing belt of Himachal Pradesh, India. Leaf samples of apple from Chamba, Salooni, Tissa, Shimla (zone III); Solan, Bajaura, Mandi, Sirmour, Palampur (zone II) and Pangi, Lahul-Spiti, Kinnaur (zone IV) were collected during the month of April-early May. Presence of major viruses on apple *viz. Apple Chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV) and *Prunus necrotic ring spot virus* (PNRSV) was detected by ELISA. Though some samples showed mixed infection of two of the tested viruses, ACLSV came across as a major virus infecting apples in the state. Primers were designed for full coat protein amplication (Accession numbers AM490253 and AM490254) using already available sequences. The PCR conditions were standardized and an amplification product of ~800bp from Kinnaur (AM408891), Solan (AM494507), Tissa (AM494511), Salooni (AM494513), Kotgarh (AM409322), Sangla (AM494512), Nagri (AM494505), Nihari (AM494508), Kalpa (AM494509), Dobi (AM494506), Bajaura (AM494510) and Palampur (AM494514) was obtained. ACLSV is also known to infect fruit trees of pear, peach, quince, plum, cherry, apricot and many ornamental rosaceous species throughout world. The virus spreads due to mechanical inoculation and different horticultural practices like grafting and pruning. Extensive surveys were also conducted for stone fruits to detect ACLSV. Among other hosts Himalayan wild cherry from Palampur (AM498044), peach (AM498050), almond (AM498046), apricot (AM498045) from Solan, peach (AM498047), wild apricot (AM498048) from Bajaura and quince from Salooni (AM498049) also gave the desired amplification in RT-PCR. Most of the sequences submitted show percent identity ranging from 91-100 and 83-100 at amino acid and nucleotide level respectively. Phylogenetic relationship in comparison to sequences available show that though values of bootstrap separation in some of the ACLSV isolates from India are very high, most of the Indian isolates fall in group A though in different sub-groups. The presence of virus has been detected by DAC-ELISA in clonal rootstocks of apple (M7, MM111, MM106) and commonly used seedling rootstocks of stone fruits (wild Himalayan cherry, wild apricot). The infected scion and rootstocks present a dangerous proposition to virus spread. Identification of healthy mother plants by proper indexing, establishment of virus-free budwood banks and use of clean horticultural practices would be worthwhile to prevent grave losses that ACLSV has been reported to cause. There would also be need of proper and strict quarantine measures to check the import of virus infected stone or pome fruit budwoods and their rootstocks.

## PP-2_47: Dissemination of viruses of cereal crops in agrocoenosises of Ukraine

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During 2002-2006 we have carried out the investigation of cereal crops in the agrocoenosises of 14 regions of Ukraine for the presence of viral infection of grain crops using enzyme-linked immunosorbent assay (ELISA) with commercial antisera (Loewe Biochemica, Munich and DSMZ, Braunschweig, Germany) specific for *Wheat streak mosaic virus* (WSMV), *Brome mosaic virus* (BMV), *Brome streak mosaic virus* (BStMV), *Barley yellow dwarf virus* (BYDV-PAV), *Wheat dwarf virus* (WDV), *Barley stripe mosaic virus* (BSMV), *Barley mild mosaic virus* (BaMMV), *Barley yellow mosaic virus* (BaYMV), S*oil-borne wheat mosaic virus* (SBWMV), S*oil-borne* c*ereal mosaic virus* (SBCMV) and *Wheat spindle streak mosaic virus* (WSSMV). The BYDV-PAV was detectable almost in all investigated regions with different infection intensities of plants in the several years. WSMV was mostly detected in Vinnytsya and Poltava regions in 2003-2004 and in Kharkiv region in 2006. BMV was found in Vinnytsya, Kyiv and Cherkassy regions during 2002-2004, and in 2005-2006 BMV was almost undetectable. From year to year WDV was identified in Kharkiv and sometimes in Kyiv region. The BSMV was identified in cereal crops in agrocoenosises near Poltava and Kyiv regions in 2003 and 2006 in Vinnytsya region in single cases only. Anymore the soil-borne viruses of cereals WSSMV and SBCMV were detected in the agrocoenosises of Ukraine for the first time in winter rye in 2004. The verification of the occurrence of both viruses was performed using DAS-ELISA with specific antisera. The virus-infected rye roots were contaminated with resting spores of the fungal virus vector *Polymyxa graminis*. The SBCMV is widespread in Europe and constitute a considerable danger for the cultivation of grain. On this account 107 species and breeding lines of grain crops and wild species of cereals were screened for resistance to this virus. The different genotypes were implanted in infected soil contaminated with SBCMV and incubated for about 3 months under climatic chamber conditions. According to the results obtained from ELISA testing 6 wheat species resistant to SBCMV were detected which can be recommended as resistance donors for the development of new cultivars. Results of ELISA were supported by the electron microscopy investigations and biological testing of viruses which could be transmitted in a mechanical way. On the basis of this experimental work a map of the propagation of different cereal viruses in the main seed-sowing regions of Ukraine was created.

## PP-2_48: Quantifying the temporal and spatial dynamics of plant viruses: a quadrat-based approach

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One of the challenges in developing cost-effective integrated disease management programs is developing the capability to accurately quantify disease/pathogen populations in space and time, and then relate this information to the amount of crop damage that results. A quadrat-based method was developed to quantify both the temporal and spatial spread of *bean pod mottle virus* (BPMV) in soybean (*Glycine max*). Soybean quadrats were established approximately two weeks after soybean emergence in soybean plots that were 6 rows wide x 7.5 m long. Quadrats were 30-cm in length (along the rows) and each quadrat consisted of four soybean plants (6 rows x 25 quadrats per row = 150 quadrats per plot). In an attempt to affect the temporal rate of BPMV development in the field, four treatments were used 2006 and five treatments were used in 2007. These were: (1) BPMV inoculated, (2) two foliar insecticide applications (3) BPMV inoculated and two foliar insecticide application, (4) an insecticide seed treated and (5) non treated control. In both years, treatments were replicated three times and were arranged in a randomized block design. Quadrats were sampled by picking the youngest fully-developed trifoliate leaflet from each of four plants within a quadrat. Sampling began 25 days after planting and was repeated at 8-10 day intervals until the crop was mature (late August). Leaf sap was extracted from each 4-leaflet (bulked) sample obtained from each quadrat and sap was then tested for presence of BPMV by ELISA (Agdia, Elkhart, IN). Quadrat position (plot, row number, and quadrat number) and the date of sampling that each quadrat first tested positive for BPMV was noted and mapped. *Bean pod mottle virus* was detected as early as the first sampling date (30 May 2006 and 12 June in 2007). The rate of BPMV quadrat infection in 2006 ranged from 0.09 to 0.17 *logits*/day, indicating that BPMV incidence was doubling every 4.1 to 7.7 days. Plots that had the earliest onset of BPMV also had the highest BPMV incidence at the end of the growing season ($R^2$ = 76.9%). Spatial analyses using both ordinary runs and black and white joins revealed clustering of infected quadrants (plants) throughout the growing season. Spatial patterns of infected soybeans were successfully detected, mapped, and analyzed using this quadrat-based sampling method.

## PP-2_49: Transmission and physical properties of virus causing sunflower necrosis

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Sunflower is the most important oilseed crop next to groundnut in India has been found to be infected by different viruses. Virus isolates *viz*. Isolate-N (I-N) and Isolate-Y (I-Y) under investigation were isolated from naturally infected sunflower crops and have been found to cause necrosis and yellowing diseases in sunflower, respectively. Transmission studies on these isolates revealed that the virus causing sunflower bud necrosis was moderately sap transmissible from sunflower to sunflower and to other hosts. This disease was found to be transmitted by vector *Thrips palmi* in presence of infected pollen from sunflower to sunflower, while it was not transmitted by seed and cleft grafting. Physical properties like Thermal Inactivation Point (TIP), Dilution End Point (DEP) and Longevity (LIV) *in-vitro* revealed that the virus isolates were inactivated between 50 to 55$^0$C and between dilution of $10^{-2}$ to $10^{-3}$. The isolates were viable up to 5 hours at room temperature of 20 to 30$^0$C. Depending on symptoms, host range, transmission and symptomatological studies virus is tentatively identified as *Tobacco streak virus* (TSV).

## PP-2_50: Development of diagnostic assays for the detection and identification of parental and recombinant PVY RNAs

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Sequence variability of *Potato virus Y* (PVY) is a distinctive feature of this potyvirus. The ability of PVY to recombination events was used to develop and validate an experimental system in order to detect, measure and compare virus recombination frequency in a transgenic and non-transgenic potato plant. It is known that possible recombination breakpoints for $PVY^{NTN}$ are located in regions between 5'-NTR-P1, HC-Pro-P3, CI-VPg and CP-3'-NTR. According to this, co-infection experiments were designed utilizing two recombinant PVY-strains: $PVY^{N}$ (CH605) and $PVY^{N}$-Wilga ($LW^{O}$). Isolate $LW^{O}$ has the molecular structure of $PVY^{N}$-Wilga, but biologically behaves like $PVY^{O}$. Comparison of their sequences allowed to propose, that the cross-over event could be situated in a region ranging from 5715 to 9170 nt. Potato transgenic and non-transgenic plants infected with a mixture of both strains were tested in IC-RT-PCR with a strain non-specific primer pair amplifying the region 9100-9400. Amplicon was cloned in pGEM-T vector and nucleotide sequences of clones obtained were analysed and compared with parental sequences. Among analysed clones those were identified that showed genomic fragments similar to both either CH605 or $LW^{O}$, but also a clone, that revealed a recombinant sequence, corresponding to a $PVY^{NTN}$ strain. The recombination appeared in the non-transgenic plant, while in the transgenic no exchanges between two parental PVY genomes were observed. To enhance probability of detection of recombinants temporal temperature gradient electrophoresis (TTGE) coupled with sequencing of the products of the electrophoretic division was chosen. This proved to be a convenient method for the detection of possible genetic exchanges between different PVY strains. TTGE makes possible the efficient visual mutation detection in the RNA target. The mutant PCR fragments can be detected by different migration distances of the recombinant and wild-type DNAs in a polyacrylamide gel containing a constant concentration of urea while the temperature is increased gradually and uniformly. Three different primer pairs were tested in order to choose the appropriate variants for this purpose. They were: PVY3'-9430/PVY5'-9100, PVY3'-5800/PVY5'-5400 and PVY3'-5740/PVY5'-5400. Test system was established on recombinant $PVY^{NTN}$ strains (isolates 12-94 and Linda) and on wild-type $PVY^{N}$ and $PVY^{O}$. Only amplicons containing a recombinant junction at position 5.715 revealed a division in 8%-TTGE, while fragments from non-recombinant strains revealed only a single band. Because of its practicability and sensitivity, TTGE method can be used for the routine studies of appearance of recombinant variants of PVY.

## PP-2_51: Tobacco transgenic rootstock harboring silencing construct against ZYMV coat protein protect wt tobacco from viral infection

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*Zucchini yellow mosaic virus* (ZYMV) causes severe damages in Cucurbitaceae. The virus is transferred from plant to plant by aphids, and insecticides were found to be inefficient in preventing the virus spread. Moreover, limited sources of resistance have been identified. Post-transcriptional gene silencing (PTGS) is a sequence-specific defence mechanism that can target both cellular and viral mRNAs, and is a widely used tool for inactivating gene expression. Viral resistance through PTGS has been shown in many cases. Posttranscriptional gene silencing spreads systemically throughout individual plants in a very characteristic manner reminiscent of viral spread. This has led to the hypothesis of a systemic silencing signal that is produced in the tissues where silencing is initiated and is then transmitted to the distant parts of the plant causing specific gene silencing. The existence of silencing signal has been shown in grafted plants, whereby silencing was transmitted from silenced rootstocks to target scions. Our goal was to evaluate the possibility of inducing protection to viral infection in susceptible scions grafted on top of a transgenic virus resistant rootstock. We choose as a model *Nicotiana benthamiana* and a silencing construct targeted to the ZYMV coat protein. *N. benthamiana* leaf disks were transformed with *Agrobacterium tumefaciens* harboring ZYMV coat in a hairpin structure, including the catalase intron as a spacer. Confirmed transformants were selfed to produce the $T_1$ generation. Progenies from individual transformed plants were screened for resistance to ZYMV by mechanical inoculation with sap from tobacco plants co-infected with ZYMV and a tobamovirus cucumber green mottle mosaic virus-CGMMV that served as helper to obtain systemic spread of ZYMV. Response to inoculation was determined by ELISA and back inoculation to susceptible plants. Selected virus resistant transgenic lines, were used as rootstocks in subsequent grafting experiments. Non-transgenic *N. benthamiana* 4 weeks old seedlings were grafted on top of T1 transgenic progenies and mechanically inoculated 3 to 4 weeks post-grafting. Non-grafted tobacco controls or control scions grafted on non-transgenic rootstocks were inoculated at the same time. Non-grafted tobacco controls or scions grafted on non-transgenic rootstocks exhibited a high ZYMV infection rate of 89% and 70%, respectively. The response of scions grafted on transgenic lines may be divided into 2 groups: scions grafted onto 2 transgenic rootstock lines were fully protected against ZYMV systemic infection, while between 8-12% of scions grafted on top of the additional 4 other rootstock transgenic lines exhibited certain level of ZYMV accumulation. We show here for the first time, that transgenic rootstocks harbouring a silencing construct, can induce resistance to viral infection in susceptible scions. Experiments aimed at evaluating the rate of specific siRNAs in the different lines, and at expanding observations in other Cucurbitaceae species are ongoing.

## PP-2_52: Tests for molecular diagnosis of *Citrus yellow mosaic virus* infecting sweet orange: a step in controlling the movement of infected budwood

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*Citrus yellow mosaic virus* (CYMV) is a member of Badnavirus genus, family Caulimoviridae. Virus is a bacilliform virus measuring 30 X 130-150 nm which contains circular, double stranded DNA genome of approximately 7.5-8.0 kbp with two internal discontinuities on each strand which appear to be the priming site for DNA replication. This virus infects different citrus species like *Citrus sinensis* (Sweet orange), *Citrus aurantifolia* (Acid lime), *Citrus grandis* (Pumello) etc., Disease symptoms include mosaic on leaf, fruit, and parts of the plant. Physiological and biochemical analysis reveals the yield loss up to 77% due to virus infection in 10 year old Sweet orange orchards. The disease is a graft transmissible disorder that is a major problem in the citrus growing belts of India especially Andhra Pradesh. As sweet orange varieties are generally graft propagated in Andhra Pradesh state, there is a greater chance in moving infection widely through the budwood used in grafting. So there is a need of diagnosing the mother plant from which the budwood is selected for grafting. As immunological tests were found ineffective with this virus, we have attempted in developing molecular diagnostics for screening the infected plants such as PCR and nucleic acid hybridization tests. Attempts in amplifying the whole genome of the different South Indian isolates have been done to use the clones as probes for virus detection in infected plant samples. Primers were designed amplifying different conserved domains of the virus genome and used in PCR diagnosis of the infected plants. Amplification from the primers resulted in 2.7 kbp fragment that was used as a probe in detection of the virus by nucleic acid hybridization tests. Both $\alpha$-$P^{32}$ radiolabelling and DIG labeling of the probes was attempted in the detection. Sequence analysis of the 2.7 kbp clone showed 88% of nucleotide level homology with the existing CYMV whole genome sequence and close Phylogenetic relationship with that of *Cacao swollen shoot virus* (CSSV), a badnavirus member. Here we have analyzed the sensitivity of the molecular diagnostics in virus detection.

## PP-2_53: Identification of soybean viral mosaic disease with IC-RT-PCR method in Lorestan province

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To identify and characterize the causal agents of soybean mosaic viral diseases in Lorestan province, southwest of Iran, this study was conducted during growing season of 2004 and 2005. A total of 254 samples of infected soybean plants showing mosaic, deformation and leaf roll symptoms were collected from soybean fields. Enzyme linked immunosorbent assay (DAS-ELIZA and ACP-ELISA) technique was used to test all the samples for the presence of the following viruses, *Alfalfa mosaic virus*, *Cucumber mosaic virus, Potato virus Y, Soybean mosaic virus*, *Bean yellow mosaic*, *Bean common mosaic virus*, *Potyvirus, Tomato spotted wilt virus and Tobacco ring spot virus* and four viruses namely *Alfalfa mosaic virus*(AMV), *Soybean mosaic virus*(SMV), *Bean common mosaic virus*(BCMV) and *Cucumber mosaic virus*(CMV) were detected. In host range studies, the SMV isolate caused mosaic, deformation, leaf roll and seed discoloration in the Williams and Clark cultivars and it caused clorotic local lesion on *Chenopodium quinoa* and *C. album* but it did not infect *Nicotiana rustica* and *N. glutinosa* too. The AMV isolate caused systemic mosaic on *C. quinoa*, *C. amaranticolor*, *Petunia hybrida* and *N. glutinosa* and brown necrotic local lesions on *Phaseolus vulgaris* cv. Bountiful and *Vigna unguiculata*, but it did not infect *Cucumis sativus*, *Capsicum annuum* and *N. rustica*. The CMV isolate produced systemic mosaic symptoms on *N.rustica* and the Williams cultivar of soybean and necrotic local lesions on *Vigna unguiculata* but it did not cause any symptom on *C. sativus* and *Lycopersicon esculentum*. Back inoculation and ELISA confirmed that *C. sativus* and *Lycopersicon esculentum* were not infected by the virus. The Williams cultivar of soybean was used for SMV and AMV propagation and the virus physical purification carried out. The SDS-PAGE method was conducted using purified virus and protein of infected and healthy plants. The molecular weight of coat protein of SMV was estimated 29-30 kDa and it was confirmed as coat protein of the virus by using SMV polycolonal antibody as probe in western blot. The molecular weight of AMV was estimated 27 kDa by using SDS-PAGE method and it was confirmed as coat protein of virus by using polyclonal antibody in western blot. The IC-RT-PCR was performed by one SMV-cpr and SMV-cpf primer pair and an approximately 469bp fragment was amplified. In order to differentiate the SMV strains, primer pairs of SMV-$G_2$ and SMV-$G_7$ were used in IC-RT-PCR, none of the strains showed reaction with $G_2$ strain primers and no fragment was amplified but all of the strains amplified a 277 bp fragment with $G_7$ strain primers. The AMV isolate amplified a 700 bp fragment by using coat-f and coat-r primers of the cp gene of the virus. The CMV isolate amplified a 678 bp fragment by using Specific primer pairs CMV-f and CMV-r and the BCMV isolate amplified a 700 bp fragment by using specific primer pairs. Comparing the nucleotide sequencing of the AMV isolate with the other isolate gene bank no significant difference was relived which showed high similarity of the isolate with the gene bank isolates.

## PP-2_54: Delay of *Cucumber mosaic virus* infection in strobilurin treated tomato plants

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The possible anti-viral activity of the strobilurin fungicide pyraclostrobin, which is speculated that elicits plant defense mechanisms, was investigated in greenhouse experiments against *Cucumber mosaic virus* (CMV), one of the most serious and destructive viruses of vegetables. Tomato plants were sprayed with pyraclostrobin (formulation F 500 containing 25% active ingredient w/v, application dose 0.8 ml formulation $L^{-1}$) 24 or 48 hours before mechanical inoculation with the virus. Plants were monitored for virus infection at regular intervals by ELISA testing and Western blotting. Lower disease incidence and slower development of infection was observed in the fungicide treated plants when inoculation with the virus was carried out 24 hours after treatment applications. Statistically significant differences between fungicide treated and control plants were obtained. The highest difference between treatments, in terms of percentage of healthy plants, was obtained one week after the inoculations. Specifically, it was found that i) under high/medium inoculum dynamic (inoculation of whole plantlet or cotyledons and first true leaves) F 500 increased healthy plants by 50-70%, ii) under low inoculum dynamic (inoculation of cotyledons) by approximately 28%, compared to the controls. When a second application of F 500 was carried out one week after the first, the antiviral effect was maintained, as at 9 dpi the difference between treatments in terms of percentage of healthy plants was 50%. The above mentioned results indicated that pyraclostrobin might induce defense mechanisms in tomatoes against CMV when plants were challenged with the virus shortly after the fungicide application. In the cases where inoculations were carried out 48 hours after treatment applications, no statistically significant differences were obtained between treatments. This is the first study of the possible anti-viral activity of a strobilurin fungicide in tomato plants under greenhouse conditions.

## PP-2_55: Identification and differentiation of viruses in asparagus plantings in Germany

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Asparagus is one of the most grown and economically important vegetable crops in Germany. Viruses infecting asparagus in various European countries were already described since the beginnings of the seventies of the past century mainly by different techniques as bioassays and host range studies. The present status of virus diseases occurring in *Asparagus officinalis* L. in Germany is uncertain. In order to analyze the current situation a survey was started in 2006. For this polyclonal antisera (PAs) were produced in rabbits to an isolate of *Aspargus virus 1* (genus *Potyvirus*) (AV-1/VB) maintained in the virus collection of the Institute of Epidemiology and Resistance Resources. Further antisera applied for the investigation by means of DAS-ELISA originating from the stock collection of the Institute of Resistance Research and Pathogen Diagnostics. For detection of *Asparagus virus 2* (genus *Ilarvirus*) (AV-2) a test kit obtained from Agdia (Elkhart) was used. In all tests, a very high incidence of AV-1 could be observed in field samples obtained from regions in South- and Middle-Germany. Because the assay showed no cross-reaction with healthy glasshouse grown asparagus plants the results confirmed that several fields were completely infested by AV-1 suggesting that virus transmission may have occurred mainly via asparagus cutting. A number of AV-1 isolates was obtained from several locations differing in their host range. At least two lines could be isolated which caused either a systemic infection in *Nicotiana benthamiana* or formed local lesions on the inoculated leaves of *Chenopodium quinoa*. Investigations by means of serological and electron microscopical methods revealed that potyvirus-like particles were present in both test plants. They reacted in PTA-ELISA and Western blot (WB) with a potyvirus group-specific PAs or a monoclonal antibody (MAb) and in RT-PCR using group-specific primers. Preliminary sequencing data of the two isolates AV-1/1 and AV-1/VB showed that their coat protein- (CP) and NIb- coding regions are related except the N-terminus of the CP. This suggests that at least two different potyviruses might occur in asparagus. The highest identity was found to the CP region of *Narcissus late season yellows virus*. However, an antiserum to that virus revealed no cross-reaction in WB. Beside AV-1 in several field samples isometric virus particle were observed which were identified as strains of *Cucumber mosaic virus* by means of PAs or MAbs. AV-2 could not be detected so far in the investigated samples. From one field sample a hitherto unidentified tobamovirus was isolated.

## PP-2_56: Quantitation by direct squash real-time RT-PCR of RNA targets acquired by single aphids

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TaqMan real-time RT-PCR was developed to detect and quantify RNA-targets from the nonpersistently transmitted *Plum pox virus* (PPV) and semipersistently transmitted *Citrus tristeza virus* (CTV) in aphids directly squashed on membranes without previous nucleic acids purification. The aphid species used, *Myzus persicae* Sulzer and *Aphis gossypii* Glover, are efficient vectors of PPV and CTV, respectively. A "Sun Gold" Japanese plum tree (*Prunus salicina*) infected with PPV-D and a Mexican lime plant (*Citrus aurantifolia*) CTV-T397 infected, were used as sources for the aphid acquisition-detection tests under controlled conditions. Non inoculated plants were used as healthy control in all experiments. After different access periods (5, 15, 30, 60 and 120 minutes for PPV and 1, 24 and 48 hours for CTV) aphid species were squashed individually on nylon membranes. Pieces of membranes harbouring the squashed samples were inserted into Eppendorf tubes. One hundred µl of Triton X-100 0.5 % for PPV or 100 µl of 0.1M glycine, 0.05M NaCl, 1mM EDTA buffer for CTV, were added, vortexed and placed on ice. For CTV analysis, an additional incubation at 95º C for 10 minutes was performed previous to vortex. Five µl of these extracts were directly used as template for real-time RT-PCR assays, to assess relationships between the duration of the acquisition period and the number of acquired viral RNA targets. The estimated number of PPV virions appeared to be very variable (from 54 to 6,179) and no related with acquisition periods. Nevertheless, the detection had a significant increase at 120 minutes. The percentage of amplifications of PPV virions from the total number of individual aphids tested after different acquisition periods was 15.66% (33 out of 225). CTV-targets were detected in 4 out of 40 aphids (10.0%) after the shortest access period (1 h). After longer access periods (24 h) CTV- targets were detected in 8 out of 40 analysed aphids (20.0%) and increased to 17 out of 49 analysed aphids (34.7%) after 48 h of acquisition. Differences in detection rates between treatments were also reflected in the quantitation analysis. After short access period 8,379 copies were detected. At long acquisition periods, 35,922 copies after 24 h and 38,280 copies after 48 h, were estimated. In the quantitation of CTV, the detection of encapsidated large dRNA and some stable dsRNAs in addition to virions, can not be excluded. These combined technologies (squash for sample preparation and real-time amplification) open possibilities for a better understanding of the role of vectors in spreading aphids transmitted viruses. Immobilized aphid species by squash on membranes constitutes a direct sample preparation method without extract preparation or RNA purification. This system allows the reliable and sensitive detection and quantitation of PPV and CTV targets in individual aphids.

## PP-2_57: Role of stage of plant infection by *Bean common mosaic virus* on yield and further seed transmission of the virus in black gram and green gram

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*Bean common mosaic virus* (BCMV) is one of the important viral diseases of black gram [*Vigna mungo* (L.) Hepper] and green gram [*Vigna radiata* (L.) R. Wilczek], which is seed and aphid-transmitted. The stage of plant infection often governs the further spread of the virus in progeny seeds. Therefore, experiments of inoculation were conducted on two different stages of plant growth i.e. before and during/ after flowering in two cultivars each of black gram and green gram. During *Kharif* 1999, fifteen days and 40 days old seedlings of two cultivars of black gram (LBG-20 & T-9) and two of green gram (K-851 & ML-267) were sap-inoculated i.e., before and after flowering, respectively. Seeds of black gram (LBG-20) and green gram (ML-267) collected from the plants that were inoculated before flowering were further sown in *kharif* 2000 and were sap-inoculated before flowering. Interesting findings were observed on appearance of symptoms on the leaves, on the seeds after harvest and on the rate of seed transmission of the progeny produced. In general all cultivars of black gram and green gram showed symptoms of leaf rolling, mosaic, leaf distortion and leaf broadening. The symptom of dwarfing was found to be more pronounced in plants inoculated before flowering in both the crops. Besides, vein necrosis was observed as more pronounced in both green gram cvs. K-851 and ML-267 in case of plants inoculated before flowering. Split seed coat was observed in the harvest collected from plants inoculated both before and after flowering in both the crops. Enzyme-linked immunosorbent assay (ELISA) of green gram seeds showing different symptoms confirmed for the first time that split seed coat symptom is associated with BCMV infection. ELISA testing of green gram (K-851 and ML-267) seeds collected from plants that were inoculated before flowering during *Kharif* 1999 showed the presence of BCMV in testae. However, black gram (LBG-20) and green gram (ML-267) seeds collected from plants inoculated in before flowering during *Kharif* 2000 showed the presence of BCMV in both testae and embryo (embryonic axis + cotyledons). Electron Microscopy further revealed the presence of flexuous particles measuring 700-900 nm. In case of black gram cv. LBG-20, seed transmission rate in the progeny was higher (24.44%) in plants inoculated before flowering than the plants inoculated after flowering started (15.59%). Significant seed yield loss per plant was observed (57.49 - 65.91%) during *Kharif* (1999 and 2000). Similar observations were made in case of green gram cv. ML-267. The percent yield loss due to inoculation after flowering was found to be less than that of inoculation before flowering. The results revealed that timing of infection of the mother plant is crucial for determining the rate of seed transmission and yield loss in the progeny. The results highlight the economic importance of early infection of BCMV in these crops.

## PP-2_58

### First sequence based evidence for occurrence of a recombinant *Begomovirus* and a satellite β-DNA associated with leaf curl disease of kenaf (*Hibiscus cannabinus*) in northern India

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Kenaf a bast fiber crop has tremendous potentiality in medicinal and paper industry. In recent years a disease causing leaf curl symptoms on kenaf has been observed in different parts of India. The infected plants showed curly leaves at early stages, and then gradually became distorted and puckered. The height of the infected plants was also progressively reduced. A survey was carried out at three villages located in the Bahraich district of Uttar Pradesh, India which revealed average disease incidence, disease severity and height reduction as 22%, 66%, and 25%, respectively. Total DNA obtained from infected leaves gave a strong Southern hybridization signal with *Mesta yellow vein mosaic virus* DNA A (EF428256) probe but the DNA from healthy samples did not give any hybridization signal. Using PCR with universal DNA β and coat protein primers, a 1.3 kb fragment corresponding to DNA β and 0.77 kb fragment corresponding to the coat protein gene of DNA A were amplified from DNA samples obtained from plants showing leaf curl symptoms while such bands were not observed with healthy control. These amplicons were then cloned and sequenced. Initial BLAST analysis with CP gene sequence (EF620563) followed by pairwise sequence identity revealed that it shared maximum 92% sequence identity with Yunnan isolate of *Malvastrum yellow vein virus* (MYVV-[Y47], AJ457824). Indepth analysis with 200 nucleotide each from N and C terminal region and core CP gene (371 nucleotide) indicated that while core CP and C terminal region showed similarity with MYVV-[Y47], the N terminal 200 nucleotide showed high similarity with *Cotton leaf curl Bangalore virus* (CLCuBV, AY705380) (96%) and Basirhat isolate of *Mesta yellow vein mosaic virus* (MeYVMV-[East Ind:Bas], DQ298138)(95%) and thus indicated the recombinant nature of the coat protein gene. Sequence analysis of complete β-DNA (1343 nucleotides)(EF 620566) revealed that while complete β-DNA shared highest (85%) sequence identity with an Indian isolate of *Cotton leaf curl Multan virus*-associated β-DNA (CLCuMB-[IN:Dab2:95], AJ316038), the satellite conserved region [SCR (1-193 nucleotide)] and βC1 ORF (549-194 nucleotide) shared 85% and 91% identity respectively with *Cotton leaf curl Rajasthan virus*-associated DNA beta (AY795608) (CLCuRB[Ind:His:06]) and the AT-rich region (700-1000 nucleotide) shared 77% identity with *Mesta yellow vein mosaic virus*-associated DNA beta (DQ298137) (MeYVB[EatInd:Bas:05]). Study thus indicated that this β-DNA also evolved as a recombinant molecule and constitutes the first report of sequence based evidence for occurrence of a recombinant *Begomovirus* and satellite β-DNA associated with leaf curl disease of kenaf in northern India.

## PP-2_59: Different virus and virus like diseases infecting *Lilium*, Tulip and Alstroemeria in Himachal Pradesh, India

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Modern cut flowers *Lilium*, Tulip and Alstroemeria are the recent geophytes introduction and are fast emerging as the most promising ornamental crops grown on commercial basis for cut flower production in different districts of Himachal Pradesh. Susceptibility of these geophytes to number of viruses has driven the flower industry to the backseat and forced commercial units around the globe to alter their flower and bulb production strategies. The history of commercial flower production clearly indicates that they have always been subjected to infections by many viruses and virus like diseases. Two virus diseases LSV and CMV were reported to infect theses crops alone or in combination by serological and molecular detection method using RT-PCR. RT-PCR analysis for samples of plants showing different symptoms detected two viruses. An amplification of size ~ 875bp with LSV specific primer pair was obtained and was confirmed as Lily symptomless *carlavirus* and an amplified PCR product of ~540bp with CMV specific primer pair was obtained confirming the identity as cucumber mosaic *cucumovirus*. Fluorescent microscopic studies indicated the association of phytoplasma with the diseased plants. Nested PCR assays by using phytoplasma specific primer pairs resulted in an expected PCR product of 1.2Kb.

## PP-2_60: Epidemiological studies of different meteorological factors and vector population in the development of virus disease(s) in bell pepper and its management using cultural practices

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Bell pepper (*Capsicum annuum* L.) is one of the most important commercial vegetable crop belonging to family solanaceae. This crop having great interstate export potential is a victim of many plant pathogens and suffers great losses not only in H.P. but worldwide due to virus diseases. All the bell pepper cultivating localities in Himachal Pradesh have been found to suffer due to infection of virus(es) on this crop with a disease incidence as high as 100 per cent. Correlation studies of disease incidence with mean air temperature, average relative humidity, wind velocity, sunshine hours and cumulative rainfall indicated that simple and partial correlation coefficients of disease incidence with vector population and relative humidity were significant during the course of investigations. Multiple correlation coefficients between disease incidence and group of independent variables worked out to be 0.97 and 0.88 during the cropping seasons respectively indicating thereby that the interaction between plants and virus(es) was greatly influenced by these factors. Early plantation of bell pepper on 9$^{th}$ April during the cropping seasons resulted in recording lower disease incidence than the later dates of planting. However, maximum yield/plot was obtained in bell pepper crop transplanted on 9$^{th}$ May each year. Maximum yield was obtained when the plants were raised at 50x50 cm spacing during both the years of experimentation. Amongst the different mulches tested, silver (polyester sheet) followed by white polythene mulch proved better than the yellow, black, blue and green coloured mulches. Planting of maize as a barrier crop was also found effective in reducing incidence of disease and increasing the yield of bell pepper. An integrated disease management experiment viz., early transplanting, plant spacing and white polythene mulch resulted in minimum disease incidence when all the three treatments were combined together in comparison to other treatments.

## PP-2_61: Selective targeting of RNAi pathyway by viral suppressors

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Viral suppressors target RNAi pathways and determine plant development. RNAi pathway operates through two classes of small RNAs, small interfering RNAs and micro RNAs. They interfere with normal gene function through RNAi machinery operating at different levels involving variety of enzyme complexes resulting in posttranscriptional silencing and translational repression. Small RNA binding characteristics does not truly reflect the interference of these suppressors in plant development. They might be affecting various enzyme complexes of RNAi machinery and indirectly affecting small RNA metabolism, which is reflected in plant growth and development. Helper component protease (HcPro), a potent RNAi suppressor from viruses belonging to *Potyviridae* family, is the most studied viral suppressor. It is a cytoplasmic protein that inhibits the initiation and maintenance steps, upstream of siRNA production. However, HcPro suppression of miRNA silencing is associated with an increased accumulation of miRNA duplex, suggesting that HcPro may act downstream of the production of miRNAs. HcPro from different potyviruses is a highly differential in its behavior in regulating RNAi pathway. *Papaya Ring Spot Virus* a *potyviridae*, possess an HcPro that shows sequence similarity with the PVY HcPro. Its non-small RNA binding ability shows its indirect involvement in RNAi pathways. Although over expression of HcPro in *Nicotiana tabbacum* causes slight phenotypic aberrations suggesting its interference in miRNA regulated plant development. Another suppressor the *cucumoviral* 2b protein from different strains of CMV, commonly known as pathogenicity factor is showing differential behavior in RNAi pathways. The 2b protein prevents the PTGS in newly emerging tissues by inhibiting long-range PTGS-signaling activity. *Cucumoviral* 2b protein from CMV- New Delhi belongs to subgroup I showing the presence of two NLS I & II that is essential for its localization into the nucleus. Constitutive expression of 2b in *Nicotiana tabbacum* causes mild phenotypic defects, which might be due to its interaction with AGO I (as similar to CMV- *Fny*) or binding with small RNA (as reported in CMV95R). The *geminiviral* AC4 protein is a very less deciphered suppressor, causing severe phenotypic aberrations in different host. Very few *geminiviral* AC4 protein were reported having RNAi regulating property. The over expression of ToLCNDV- AC4 causes severe phenotypic abnormalities in *Nicotiana tabbacum*, *Nicotiana benthamiana* and *Solanum lycopersicon*, suggesting its probable role is micro RNA regulated pathways.

## PP-2_62: Sequence diversity in the coat protein (CP) gene of *Papaya ringspot virus* and development of CP gene constructs for papaya transformation

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*Papaya ringspot virus* (PRSV), a member of the family *Potyviridae*, genus *Potyvirus*, causes one of the most economically important papaya diseases worldwide including India. The disease is characterized by mosaic, distortion and shoe-stringing of leaf lamina, watersoaked oily streaks on the petiole, trunk and ringspot on fruits. Both conventional and genetically engineered (transgenic) resistance strategies have been successfully deployed to manage PRSV in papaya. Success of both the resistance strategies depends on the availability of information on genome organization and genetic diversity of PRSV population. Diversity in the PRSV isolates was analyzed by amino acids sequences comparison of coat protein of 28 isolates originating from five different geographical locations. The isolates from different locations were highly heterogeneous in CP length (275-289) and amino acids sequences (up to 23%). Maximum heterogeneity was observed in southern isolate (up to 23%) followed by central (up to 11%) eastern and northern (up to 10%) and western (up to 7%) isolates. Though PRSV isolates were grouped into three clusters, there was lack of relationship between diversity and geographical origin of the isolates. The existing profile shall have strong influence on the management of PRSV through transgenic resistance. To confer resistance, gene constructs targeting the complete (~850 bp) or conserved region of CP gene (~410 bp) were developed in pBinAR. The CP gene constructs in *E. coli* and *A. tumefaciens* have been used for transformation to generate virus resistant transgenic papaya.

## PP-2_63: Survey and serodiagnosis of viruses infecting papaya (*Carica papaya* L.) plants in Uttar Pradesh, India

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Papaya (*Carica papaya* L.), is an important fruit crop, grown throughout the tropical and subtropical countries of the world. India is the second largest producer of papaya fruits. Although, the total area under cultivation has recorded a regular increase in the recent past, yet, fruit production has not shown corresponding increase. Low productivity of papaya is mainly due to the losses caused by various biotic factors including viral diseases. Among viral diseases papaya ring spot and leaf curl are the major diseases which cause considerable losses in yield and quality of fruits. Incidence of papaya ring spot virus disease ranged from 45to 85% and 47 to 90% while, leaf curl disease incidence ranged from 17 to 30% and 16.00 to 36% during 2004-06 and 2005-06, respectively. Minimum ring spot and leaf curl disease incidence was recorded in Varanasi district followed by Faizabad, Lucknow and Etawah during both years. Most of the papaya plants surveyed showed characteristics symptoms of papaya ringspot virus and papaya leaf curl virus. Serodiagnosis studies on the virus under study by direct antigen coating (DAC)-ELISA have revealed that out of seven polyclonal antisera, only polyclonal antiserum of *Papaya ringspot virus* (PRSV) reacted strongly with virus antigen, while antiserum of *Watermelon mosaic virus* showed weak reaction. These results confirmed that the causal virus was a strain of PRSV. On the other hand sixty four samples were tested for the presence of PRSV and *Papaya leaf curl virus*, 52 samples confirmed infection of PRSV and 12 samples with *Papaya leaf curl virus*.

## PP-2_64: Biological, serological and molecular characterization of chrysanthemum isolate of *Cucumber mosaic virus*

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Garden chrysanthemum (*Chrysanthemum morifolium* Ramat., family *Asteraceae*) is a popular ornamental plant grown worldwide for its beautiful bloom of various shape, color and excessive long vase life. *Chrysanthemum morifolium* plants showing ring spot symptoms were observed in gardens and nurseries of Lucknow during the winters of 2004-2006. The disease was mechanically transmitted to indicator species including systemic host. Leaf dip preparations revealed the presence of 29 nm isometric core particles in infected chrysanthemum and mechanically transmitted *Nicotiana tabaccum* cv. White Burley plant, intimating the presence of Cucumovirus. Indirect enzyme linked immunosorbent assay (ELISA) carried out with CMV antiserum (PVAS 242a, ATCC, USA) resulted in the strong serological reaction and showed OD 0.385±0.002 at $A_{405\ nm}$ (as compared to positive control OD 0.323±0.005) on the background of negative control (0.076±0.003). Infection of CMV was further confirmed by reverse transcription-polymerase chain reaction (RT-PCR) using complete RNA 3 gene specific primers. RT-PCR resulted in the amplification of ~2.2 bp amplicon, which was transferred to nylon membrane and hybridized with CMV specific probe to confirm the identity of amplicon showed positive signals. The PCR product was gel purified, cloned, sequenced and the sequence data was submitted to GenBank (EF153733). Sequence alignment of complete RNA 3 component of chrysanthemum isolate using BLAST analysis showed maximum 99% identity with CMV strains of amaranths, jatropha, rauvolfia and datura while minimum 95-93% nucleotide identity was obtained with banana and tomato isolate, respectively. During phylogenetic analysis, the chrysanthemum isolate under study showed closest phylogenetic relationships with the CMV isolates reported from India and clustered with them, on the contrary it showed distant relationships with the TAV and PSV isolates, considered as out group for the present study. It also showed distant relationship with the CMV strains belong to Subgroup IA and II. Sequence alignment and phylogenetic analysis contributed that the chrysanthemum isolate is an isolate of *Cucumber mosaic virus*, belongs to subgroup IB.

## PP-2_65: Epidemiology of virus causing necrotic wilt causing sunflower (*Helianthus annuus L.*)

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Investigation on virus causing necrotic wilt of sunflower were undertaken as the disease is of common occurrence in Marathwada region. The virus isolates viz. Isolate N (I-N) and isolate (Y-I-Y) eluted from naturally infected sunflower plants were tentatively identified as Tobacco Streak Virus (TSV) depending upon symptomatalogy reaction on diagnostic host, transmission, physical properties and host range studies. Considering the importance of disease, epidemiological studies undertaken revealed that the hosts are supposed to act as source on inoculum and plays a major role disease epidemics. Correlation between vector *Thrips palmi* and necrosis disease was positively significant and distribution of sunflower plants infected by necrosis and yellowing disease were found to be random, indicating that the diseases were of simple interest. The disease with necrotic symptom is predominant in *kharif* while yellowing is predominant in *rabi* and incidence was found to be dependent on activities of vector.

## PP-2_66: Incidence of *Tobacco streak virus* on Bt. cotton under natural condition

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Cotton is an important fibre crop. Cotton streak disease was newly recognized in India, identified to be caused by the *Tobacco streak virus* (TSV). The virus was recognized on 26 coded Bt entries at Kok village, Jintor talaq in Parbhani District, Maharastra, and Cotton Research Station, Mehboob Bagh, Marathwada Agricultural University, Parbhani. Incidence of TSV was noted for the first time in Maharashtra. Disease observations were recorded from July-06 to November-06 at an interval of 15 days. Data revealed that viral disease incidence was recorded at increasing rate up to the end of September 06 and further incidence was noted in decline rate from October to November 2006. Maximum disease incidence was recorded in the month of September observation in the range of 36.59 to 83.88%. Lowest incidence was noticed on entry 6108 and highest on entry 6126.

## PP-2_67: Integrated management of *Papaya ringspot virus* (PRSV) in Peru

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The papaya crop is traditionally planted in small field lots and it is considered the "poor person's crop" in the jungle region of Peru. Papaya virosis is a severe problem affecting the main papaya growing areas in Peru, causing adverse social and economic effects in the local population. The disease reduces the crop yield over 40% and affects the quality of the fruits. An integrated management was initiated with the participation of CIP, INIA, and SENASA to reduce disease incidence in the main papaya producing areas. Serological and host-range tests were used to identify virus in papaya fields of the Chanchamayo and Perene valleys in the Junin Region. Weeds in or close to papaya fields were collected for identification and testing for PRSV using ELISA. Up to 100% PRSV incidence in papaya fields was recorded and PRSV was identified as the main virus affecting papaya. An antiserum specific for the detection of PRSV was produced and some wild alternate hosts of PRSV identified. Studies of disease infection gradients in the Perene valley suggest that virus infection originates from inside the papaya fields. Therefore, infected weeds may play an important role in the dissemination and prolongation of the disease. Aphids, (mainly winged forms) though very rarely seen on papaya plants, are the vectors that transmit PRSV. Five species of aphid were found in traps placed in papaya fields. Epidemiological studies suggest that aphids acquire the virus and inoculate papaya via alternate hosts that play an important role in the dissemination and prolongation of the disease. Therefore, elimination or reduction susceptible weed presence to act as a virus inoculum source can be an important factor to help control the disease. Planting a covering crop unsusceptible to the virus could be used to retard the development of susceptible weed plants and help render non-viruliferous vectors. A technological package to control PRSV (covering crops, manure application, and transplanting 3-4-month virus-free papaya plants, natural barriers, isolation -200 m- from other papaya fields) to control PRSV was placed in pilot areas (Shincayacu, San Ramon, and Pichanaki in the Junin Region). Results have demonstrated that the use of the technological package has delayed the virus infection by over 3 months, allowing a better commercial production. Inclusion of "mild" strains of PRSV (for cross-protection) and tolerant varieties into the technological package are now under evaluation.

## PP-2_68: *Chickpea chlorotic stunt virus* affecting cool-season food legumes in West Asia and North Africa

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Viruses causing yellowing/stunting symptoms are the most important virus diseases affecting cool-season food legumes (faba bean, chickpea and lentil) in West Asia and North Africa (WANA), and in some years these viruses have caused almost complete failure of chickpea and faba bean crops. Surveys conducted in WANA showed that there are at least 6-8 viruses that produce similar symptoms (mostly leaf-rolling, yellowing and stunting of infected plants) in food legume crops. A large number of samples with yellowing/stunting symptoms were collected during the last decade and tested using both (i) the serologically-based tissue blot immunoassay (TBIA) technique using a battery of specific monoclonal and polyclonal antibodies and (ii) the molecular-based polymerase chain reaction (PCR) using specific primers for viruses that belong to the families *Luteoviridae* and *Nanoviridae*. Results showed that the viruses causing yellowing/stunting in cool season-food legumes in WANA are mainly two ssDNA viruses (*Faba bean necrotic yellows virus* and *Chickpea chlorotic dwarf virus*) and three ssRNA viruses (*Bean leafroll virus*, *Beet western yellows virus* and *Soybean dwarf virus*). Large numbers of plants with yellowing symptoms did not react with either specific monoclonal antibodies or with specific primers. Further testing (serology, PCR and aphid transmission) showed that most of these plants were infected with *Chickpea chlorotic stunt virus* (CCSV, genus *Polerovirus*: family *Luteoviridae*) and transmitted mainly by *Aphis craccivora* Koch in a persistent manner. The incidence of CCSV was higher than that of other viruses and exceeded 50% in some chickpea and faba bean fields in countries such as Syria, leading to high yield loss. CCSV has been recently reported only in Ethiopia, and this is the first report of CCSV in other countries within WANA region. The use of PCR technology and sequencing with reference to detection and characterization of CCSV will be presented.

## PP-2_69: Breeding for resistant varieties to control rice virus disease in Korea

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There are three kinds of virus diseases in Korea; *Rice stripe virus* (RSV), *Rice dwarf virus* (RDV) and *Rice black-streak dwarf virus* (RBSDV). The early virus disease outbreak was reported in the 1940s in major rice growing areas of southern regions in Korea. In 1960s and 1970s, more than 50% of total rice growing area was infected these rice virus disease and it led yield to reduce highly. The use of resistant variety is the most economic and environmentally soundest approach for protecting rice crop against viral disease damage. Thus, breeding for rice virus resistance is one of the major objectives of the rice breeding program in Korea. To breed virus resistant rice variety, developing an efficient screening method is the most important. We developed mass screening method using net-house and MAS (marker assisted selection) system using DNA marker linked to virus resistant gene. With these methods, we can evaluate as many as 1,500~2,000 pedigree lines in one time with high accuracy. Since the developing of the first rice RSV resistant variety “Nagdong” in 1975, many resistant varieties to RSV were breed out in Korea. These varieties were observed to be the most effective control measure of RSV disease in Korea ecosystems. Early breeding objectives were directed toward developing rice varieties with good plant type, high yield and resistant to disease and pests. But recent days, most farmers prefer to cultivate the cultivars with high grain quality, though they are resistant to virus disease or not. Continuous planting of these susceptible cultivars and climatic conditions favorable for vector insects of virus disease were the main factors of recent virus disease outbreak in Korea. Thus, the breeding objectives for virus resistance in recent were focused on grain quality and environment friendly in Korea. We started a program to breed virus resistant variety with high grain quality and multi resistant to virus disease and pests such as brown plant hopper and green rice plant hopper.

## PP-2_70: Frequency of mixed infection of *Tobacco streak virus* and *Peanut bud necrosis virus* in groundnut

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Bud necrosis disease (BND) first recognized in 1968, caused by the *Peanut bud necrosis virus* (PBNV; *Tospovirus*) is an important constraint to groundnut (*Arachis hypogaea* L.) production in Southeast Asia, including India. BND symptoms on groundnut include mosaic mottling symptoms on leaves, drooping of petioles followed by terminal bud necrosis. Early infection causes stunting and proliferation of axillary shoots. In 2000, a sever epidemic of BND-like disease was observed in groundnut crop grown in over 250,000 ha in Anantapur district of Andhra Pradesh, India. Symptoms of affected plants were similar to that of BND except that necrosis on petioles and stems were very pronounced and plants infected at early stage died prematurely. Initially it was assumed that the PBNV or a new strain of it as a cause for this severe epidemic. Subsequent studies confirmed that *Tobacco streak virus* (TSV; *Ilarvirus*) was responsible for the new disease outbreak on groundnut and it was named as stem necrosis disease (SND). Surveys were conducted in groundnut fields during 2001-04 in Anantapur district by randomly collecting leaf samples from diseased plants from several different farmer fields and tested them for PBNV and TSV by enzyme-linked immunosorbent assay (ELISA). This has demonstrated occurrence of both PBNV and TSV in different proportions. Surprisingly, the incidence of mixed infection of PBNV and TSV was very low. Symptoms in plants infected singly with TSV or PBNV or with both the viruses were almost similar and difficult to distinguish. Of 175 symptomatic plants from rainy season crop (Jun-Nov) tested during 2000, 57.1% were positive to PBNV and 42.8% were positive to TSV. In the same year most of the parthenium (*Parthenium hysterophorus)* plants grown adjacent to groundnut fields tested positive to TSV, but not to PBNV and symptomatic sunflower plants intercropped in groundnut fields were positive to TSV but not to PBNV. In 2001, of 113 symptomatic samples tested 41.5% were positive to TSV, 30.9% were positive to PBNV and 6.2% were positive to both TSV and PBNV. Of 104 symptomatic samples tested in 2002, 43.2% were positive to TSV, 57.7% were positive to PBNV and 3.8% were positive to both the TSV and PBNV. Of 900 symptomatic samples tested in 2004, 53.9% were positive to TSV, 45% were positive to PBNV and 3.5% samples were positive to both TSV and PBNV. The incidence of these two viruses varied from field to field. In some fields PBNV incidence was high and in some other fields TSV incidence was high, but co-infection of plants with both the viruses was very low (3.5 to 6.8%) even in fields where near equal incidence of TSV and PBNV were observed. Thrips are involved in transmission of these two viruses, but transmission mechanisms are different. Several species of thrips aid in the TSV transmission apparently by mechanical inoculation in the presence of TSV-infected pollen rather than as direct vectors, while PBNV is transmitted in a persistent manner only by *Thrips palmi*. At field level abundance of inoculum sources (viruliferous thrips in case of PBNV and thrips+TSV-infected pollen for TSV) and preference of thrips species might influence the incidence of PBNV and TSV. In a scenario of abundant sources of TSV and PBNV and thrips in the same region, it is unlike that thrips selectively infect plants with PBNV or TSV. We suspect suppressor role (antagonism) of one virus on other depending on the time of infection. For example, plants infected first with PBNV may suppress multiplication of TSV inoculated subsequently, and vice-versa. We are conducting experiments to determine any synergistic or antagonistic effects of these two viruses in virus titer and symptom phenotype in groundnut. Nonetheless, from field survey data it was clear that infection with PBNV and TSV or by both results in severe symptom phenotype in groundnut and requires different management tactics to control these virus diseases in endemic locations. It is worthy to investigate this situation in other crop species which are susceptible to both TSV and PBNV in India.

## PP-2_71: Incidence of seed transmitted viruses in cowpea and soybean in Nigeria

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Virus transmission through seeds has high epidemiological significance as primary source of virus inoculum for insect vectors, which aid in further secondary spread. In addition, seed-transmitted viruses pose constraint to germplasm exchange, and if unchecked can lead to introduction of viruses into new niches. To help safe movement of healthy germplasm materials and supply of virus-free seed to farmers, we monitor for seed-transmitted viruses in cowpea (*Vigna unguiculata*) and soybean (*Glycine max* L.), two major legume crops in Africa, and also forage legume cover crops at IITA-Ibadan Station, Nigeria. We present the results of virus indexing studies carried out on 165 accessions of soybean, 1081 accessions of cowpea and 409 accessions of various forage legumes during 2004 to 2007. Seedlings were monitored for visual symptoms three weeks after sowing in the field. All symptomatic plants were collected for virus testing in leaf sap extracts (1:10 w/v) by enzyme-linked immunosorbent assay (ELISA) with respective antibodies. Leaf samples from asymptomatic plants were also collected randomly and tested for viruses. Soybean lines were tested against five viruses: *Cucumber mosaic virus* (CMV), *Tobacco ringspot mosaic virus* (TRSV), *Bean pod mottle virus* (BPMV), *Bean yellow mosaic virus* (BYMV) and *Southern bean mosaic virus* (SBMV). Of 28 lines tested in 2004, 57% samples were positive to only CMV. Of the 99 samples tested in 2005, 30%, 6% and 3% of the lines were positive to CMV, BYMV and SBMV, respectively, but TRSV and BPMV were not detected. Mixed infection with BYMV, CMV and SBMV were found in 21.2% samples tested. Of the 38 samples tested in 2006, 18% of the lines were positive to SBMV only. Cowpea accessions were indexed against five viruses: CABMV, *Cowpea yellow mosaic virus* (CYMV), *Cowpea mottle virus* (CMeV), SBMV, and CMV. In 2004, 500 seeds from each of the 141 accessions were grown-out in the field and observed for virus-like symptoms. The germination rate ranged from 5%-95%. Seven of the 141 accessions showed virus-like symptoms after germination. In 2005, a total of 556 accessions were indexed. The percentage infection was 22% for CABMV, 312% for CMeV, 10% SBMV and 9% for CMV, while 22% had mixed infection with these four viruses. In 2006, 100 seeds each from 122 accessions were grown-out in the screen house for visual observation. The germination rate ranged from 40-100% and none of the accessions showed virus symptom and they were not tested by ELISA. In 2007, a total of 262 accessions were indexed and CYMV was found in 3% samples, CMV in 4% samples, CMeV in 9% samples, SBMV in 1% samples and mixed infection of all the five viruses in 2% samples. Forage legumes (*Mucuna sp., Cajanus cajan, Centrosema sp., Crotolaria sp., Canavalia sp., Clitoria ternatea, Tephrosia brascteolata, Pueraria, Sesbania, Stylosanthes sp., Indigofera, Psophocarpus palustris, Lablab purpureus, Aeschynomene histrix* and *macrotyloma uniflorum*) used as pasture and cover crop were also indexed for viruses and pooled data are presented. In 2004, growing out test for 96 accessions of these forage legume species was carried out. The germination rate ranged between 7-99% and none of the 96 accessions showed virus-like symptoms, and they were not indexed for virus in ELISA. In 2005, a total of 262 samples were tested and 12%, 13%, 17% and 26% were positive to CABMV, CMeV, SBMV and CMV, respectively, but none of the samples were positive to CYMV. About 25% had mixed infection with the four viruses. A total of 51 samples were indexed in 2006 against five viruses (CABMV, CMV, CYMV, CMeV and SBMV). Of these 6% were positive for CABMV and CMV, and 4% for CYMV. None reacted with CMeV and SBMV while 4% had mixed infection of CABMV, CYMV and CMV. This study demonstrates high frequency of seed-transmitted viruses in cowpea, soybean and forage legumes (which would serve as inoculum sources for crop plants) and also indicates the variable incidence of different viruses in different seasons, which warrants for an integrated strategy to combat various diseases in cowpea and soybean.

# Poster Session – III

## PP-3_72: Evaluation of FHIA hybrids for resistance to *Banana bunchy top virus* and yield potential in Malawi

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One of the major banana diseases in Malawi is *Banana bunchy top virus* (BBTV) which has destroyed about 1,000 hectares of Williams and other local bananas in the country so far. FHIA hybrids believed to be tolerant to the disease and high yielding have not been tried in Malawi. A banana trial was planted at Limphasa trial site in Nkhata-Bay district, northern Malawi in April, 2004 with the main objective of assessing FHIA hybrids and other introduced banana cultivars for tolerance to BBTV and determining their yielding potential in Malawi. Five FHIA hybrids (SH 3640, FHIA 17, 21, 23 and 25), five cooking bananas (Bluggoe or Harare, Saba, Pelipita, TBMx 1378 and TBMx 5295-1) a Cavendish banana (Williams as a control), a French plantain (Nzeru) and KM 5 were planted in a two-replicated trial in April; 2004. Five mats formed a plot in each of the two replicates. Data was collected for only the first cycle because most plants had died of the disease in the subsequent cycles. Results show 90% of Williams mats (9 out of 10) were infected by BBTV against 50% and 30% for FHIA hybrids and cooking bananas, respectively. KM 5 and Nzeru had 40% of their mats attacked. FHIA hybrids produced the highest yields (27.9 kg and 28.2 kg bunch weights for FHIA 17 and 25, respectively). The average bunch weight was 16.8 kg. Cooking bananas Harare (Bluggoe), Saba and Pelipita yielded lower than the average. Williams gave 15.2 kg, TMB × 1378 (16.9 kg) and TMB × 5295-1 (19.3 kg). Other FHIA hybrids yielded as follows: SH3640 (14.4 kg), FHIA 23 (16.4) and FHIA 21 (17.8 kg). All the banana cultivars in the trial are susceptible to the disease but all cultivars other than Williams are tolerant to the disease. Williams is widely reported to be very susceptible to the disease. FHIA hybrids and cooking bananas (TBMx 1378 and TBM × 5295-1) are high yielding in Malawi. FHIA hybrids and other banana cultivars could temporarily be recommended for one cycle production in BBTV prone areas to replace Williams. However, more data would be required to fully establish the response of the banana cultivars to the disease.

## PP-3_73: Response of selected candidate resistant summer squash cultivars to *Zucchini yellow mosaic virus* (ZYMV) infection

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Recently, *Zucchini yellow mosaic virus* (ZYMV) has caused considerable losses of cucurbits grown in South Moravia and spreads gradually to northern parts of the Czech Republic. The commonly grown squash cultivars are highly susceptible to ZYMV. Characteristic symptoms on leaves of cucurbitaceous plants are yellow mosaic and deformed fruits. The virus is very variable. In the Czech Republic there were three ZYMV strains that differ in pathogenicity were known to occur. The resistance of summer squash (*Cucurbita pepo* L. convar. *giromontiina* Grebenščikov) cultivars described as ZYMV-resistant, Jaguar, Cougar and Hurakan, from the USA, was examined by artificial inoculation with a highly pathogenic Czech ZYMV isolate, ZYMV-H. Summer squash Zelená, a Czech cultivar susceptible to ZYMV, served as the control. An ELISA based diagnostics was used to evaluate the degree of resistance as the relative concentration of virus protein in leaves. The highest dilution resulting in the positive reaction in ELISA (titer) was considered as the reciprocal value of the ZYMV protein relative concentration. The titer of virus in leaves was determined every two weeks up to the seventeenth week after inoculation and tests were repeated twice with a group of ten plants per cultivar. Obtained relative virus concentrations in plants of one group at the same time were close to each other; deviations were only one dilution step. The response of all the tested cultivars to the ZYMV infection was in increasing virus concentration in leaves up to the first four or six weeks after inoculation when the highest differences in concentrations between cultivars were found. Thereafter it remained constant excluding cv. Hurakan in which the virus concentration decreased after the eighth week. In the case of the sensitive control cv. Zelená, the virus concentration in leaves during the tested period was similar to cv. Hurakan but at a much higher level. All the cultivars had the same level of virus concentration at the end of experiment; Jaguar, Cougar and Zelená even at the same value, only cv. Hurakan at the value four times lower. Of the three tested summer squashes, cv. Hurakan possessed the highest degree of resistance to ZYMV-H and it also had the least deformed fruits.

## PP-3_74: Effect of *Cotton leaf curl virus* (ClCuV) on reproductive ability of *Bemisia tabaci*

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*Cotton leaf curl virus* (CLCuV) is a group of whitefly transmitted geminiviruses that cause extensive damage to cotton crop in India and Pakistan. Begomoviruses have a complex association with their vector whitefly. The effect of plant viruses on their vector is a key to the understanding geminiviruses epidemiology and developing effective control measures. Previous studies suggest that at least some geminiviruses are reminiscent of insect pathogens and are deleterious to their whitefly vector. The objective of the present study was to determine the effect of cotton leaf curl virus (CLCuV) on reproductive capability and longevity of whitefly on cotton plants. Fecundity and longevity of viruliferous and non viruliferous whiteflies was recorded on CLCuV infected and healthy plants of cotton after 5, 20 and 35 days of inoculation. Whiteflies deposited significantly lower number of eggs on virus infected cotton plants(46.53) than healthy plants (53.20).There was no significant difference for the number of eggs deposited on 5 and 20 days old virus infected plants, whereas whiteflies deposited significantly lower number of eggs (44.83) on 35 days old virus infected plants than healthy plants of same age. Viruliferous whiteflies deposited significantly lower number of eggs than non-viruliferous whiteflies on CLCuV resistant cotton plants. Differences were significant on 35 days old plants, whereas differences were non significant on 5 and 20 days old plants. The virus infection did not affect the hatchability of the eggs on healthy and virus infected plants. Hatchability of eggs of viruliferous and non-viruliferous whiteflies was also non significant.

## PP-3_75: Negative effects of *Cotton leaf curl virus* on host suitability and longevity of *Bemisia tabaci*

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The effects of *Cotton leaf curl virus* (CLCuv) on the settling behavior of its vector whitefly, *Bemisia tabaci* (Gennadius) were examined on cotton plants under greenhouse and field conditions. Significantly lower number of whiteflies settled on virus infected plants to healthy plants of cotton under choice tests. Settling behavior of viruliferous whiteflies differed significantly within 10 minutes of beginning of the settling experiment on 35 days old virus and within 8 hours on 20 days old virus source. Settling behavior did not differ significantly on 5 days old virus source. Non viruliferous whiteflies preferred ClCuV susceptible host to CLCuV resistant host. Settling behavior differed within 2 hours on 40 days and 55 days old plants while it took 24 hours to distinguish 25 days old plants. The longevity of adult male and female whiteflies on virus infected plants was significantly lower than the longevity on healthy plants. Differences were also significant on different age (20 and 35 days) virus sources. Longevity of male and female viruliferous whiteflies (11.10 days) was significantly lower than the longevity of non virulifrous whiteflies (13.14 days). The longevity also differed significantly on cotton plants of different age groups. These results suggest that CLCuV has a negative effect on its vector and has some features reminiscent of insect pathogen.

## PP-3_76: Bud necrosis of watermelon (WBNV) and associated species complex of thrips

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*Watermelon bud Necrosis virus* (WBNV), a tospovirus is a major limiting factor in successful cultivation of watermelon in South India. Four out of 11 thrips species that vectors tospoviruses are present in India. It is not clear which of the four species can effectively transmit WBNV. Furthermore, there may be other thrips species associated with watermelon that could be potential vectors. Hence, studies to determine thrips species involved in transmission of WBNV were conducted during 2005-2006 in Karnataka, Andhra Pradesh and Maharashtra states, India. Thrips were identified as per the taxonomic key provided by Bhatti (2000).Watermelon crops were sampled through out the period for thrips (Bhatti, 2000). *Thrips palmi* was the predominant species (95%) and *Scirtothrips dorsalis* and *Frankliniella schultzei* formed only 0.5% each of the total population. Thrips catches on yellow funnel traps set up in melon field corroborated over thrips sampling on melons.

## PP-3_77: A novel tool to study the epidemiology of *Cucumber vein yellowing virus* (CVYV) and *Cucurbit yellow stunting disorder virus* (CYSDV) in cucurbit crops using a real time RT-PCR assay

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Since 1980 emerging viruses infecting cucurbits around the world have been described, some of which are arthropod-transmitted and have become widespread causing economic damage following multiplication and spread of the vectors. In the southeast of Spain, *Cucumber vein yellowing virus* (CVYV) and *Cucurbit yellow stunting disorder virus* (CYSDV) are the most economically-important pathogens of cucurbit crops and both are included in the EPPO A2 Action List of pathogens. Meanwhile virus-tolerant crops are being developed, management of CVYV and CYSDV in Spain relies on control of the vector, *Bemisia tabaci*. Numbers of whiteflies infesting a crop at an early stage of an epidemic are a measure to predict the outcome of disease but do not give information on how many of the whiteflies are actually viruliferous. Therefore, a Real Time RT-PCR assay was developed as a rapid and routine diagnostic system for the reliable detection and quantitation of both economically-important viruses in *B. tabaci*. As a quantification method it can provide useful knowledge regarding virus-vector interactions. In addition, an internal control test has been developed to avoid false negative results. *B. tabaci* adults were collected from greenhouses with cucumber, melon and zucchini crops and analysed individually. Use of *B. tabaci*-specific primers and a Taqman® probe allowed confirmation that amplifiable RNA was present in all samples. From these whiteflies, positive for CVYV were slightly greater than positive for CYSDV. Only a small percentage of individuals had double infections (CVYV and CYSDV), finding a larger proportion of insects with CVYV being co-infected with CYSDV than insects with CYSDV being co-infected with CVYV. The normalized amounts (Log MNE) of virus-specific DNA quantified in the positive whiteflies collected from the field showed a wide range of concentration. Regression analysis revealed important differences between the two viruses in relation to the crops from which the whiteflies were collected. The amounts of CYSDV detected in whiteflies from melon and zucchini were significantly higher than those of CVYV. Whiteflies collected from cucumber had higher amounts of CVYV when compared with CYSDV. The amounts of CYSDV in these latter whiteflies were also significantly less than in those found in melon and zucchini. On the other hand, levels of CVYV in whiteflies from cucumber were higher than in whiteflies collected from melon and zucchini. This novel Real Time RT-PCR Taqman assay contributes to improve and increase the available tools to study the epidemiology and virus-vector-plant relationships. This method is fast, sensitive and specific to detect these two viruses in individual *Bemisia tabaci* whiteflies collected from the field and therefore it could be included in a risk assessment procedure for CVYV and CYSDV.

## PP-3_78: Identification and ecology of thrips species, the vectors of tospoviruses in India

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At least, eleven out of 6000 described species of thrips have been worldwide, reported as vectors for Tospoviruses. India hosts around 700 species of thrips and five of these are reported vectors for Tospoviruses. All the five species, *Ceratothripoides claratris* (Shumsher), *Frankliniella schultzei* Trybom, *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny, and *Thrips tabaci* Lindeman belongs to subfamily Thripinae under family Thripidae. Till now, Western flower thrips (WFT) *Frankliniella occidentalis* (Pergande), most predominant species, worldwide, has not been reported from India. In India *Watermelon bud necrosis virus* (WBNV), *Peanut bud necrosis virus* (PBNV), *Iris yellow spot virus* (IYSV), potato stem necrosis disease (PSN), etc., are the reported tospoviruses. Besides, thrips are also associated with the transmission of *Tobacco streak virus* (TSV). While undoubtedly, the association between virus and the vector is species specific, an understanding the epidemiology of tospoviruses will be incomplete without a comprehensive knowledge of thrips identification and ecology. More than any other group, biology of thrips vectors is closely associated with acquisition and transmission of tospoviruses. Hence, the present study was carried out to provide an illustrated morphological identification key, distribution and the associated host plants for vector thrips species in India. Efforts are being made to integrate morphological and molecular systematics to make the identification speedy. *Ceratothripoides claratris* (Shumsher) is collected from two host plant, *i.e. Setaria glauca* (Poaceae) and *Clitoria ternata* (Leguminosae). *Frankliniella schultzei* Trybom on thirty host plants in fifteen families, *Scirtothrips dorsalis* Hood on forty host plants in 20 families, *Thrips palmi* Karny on twenty-eight host plants in twelve families, and *Thrips tabaci* Lindeman on five host plants in three families.

## PP-3_79: Sensitive broad spectrum detection and quantification tools for managing peanut clump disease

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*Peanut clump virus* (PCV) and *Indian peanut clump virus* (IPCV) cause significant losses in crops of groundnut. The lack of efficient control measures implies a real need for detection methods. Nevertheless, virus is often present in asymptomatic association with the numerous pecluvirus host plants. Furthermore, serological detection is hampered by the major differences among the amino acid sequences of virus coat proteins. Another reason for the need of detection methods is the fact that pecluviruses are both transmitted by seed and soil. Pecluviruses are transmitted by the soil plasmodiophorid, *Polymyxa graminis*, vector of up to fifteen soil-borne viruses of *Gramineaceae* from the genera *Bymovirus, Furovirus* and *Pecluviruses.* PCV and IPCV are also seed transmitted at rates varying from less than 48 % to more than 55% on groundnut. Up to date, no natural resistance has been evidenced for groundnut genotypes. Therefore, the availability of non-serological, sensitive and broad-spectrum detection methods are essential for the management of clump disease. RT-PCR targeting of the 3'-ends of each RNAs of the bi-partite PCV and IPCV genomes was compared to ELISA. RT-PCR proved extremely useful in the detection of the different serotypes&serogroups of the genus *Pecluvirus.* It detected viruses belonging to each of the three known serotypes of IPCV as well as PCV isolates from Burkina Faso, Mali, Niger and Senegal. It gave positive reactions with samples identified through bioassay and electron microscopy that had failed to react with any of the available antisera. The results emphasize that pecluviruses can be present in graminaceous hosts, even when no symptoms are apparent, and stress the risks of disease spreading because of seed and soil exchanges. The 3'end UTR of virus isolates was sequenced and piled up with Genbank available sequences, confirming its conservation. A primer pair and a 23 nucleotides Taqman probe were designed for quantitative real-time RT-PCR. The technique is offering a gain in sensitivity compared to RT-PCR and is useful for the evaluation of the resistance of cereal cultivars to pecluviruses. Strategies for the management of the disease using such detection tools will be discussed

## PP-3_80: A generic (RT)-PCR test for caulimoviruses

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For a considerable number of viruses from different genera sequence data are available and for different virus genera general PCR primersets have been described. Not for all economically important viruses reliable detection methods are available yet. Caulimoviruses constitute a group of DNA plant viruses that cause diseases in a wide range of crops. To only a limited number of them specific diagnostics are available. Detection of poorly characterized or new caulimoviruses is often not possible or reliable. Based on available sequence data caulimovirus genus-specific primers were designed The primers were tested to available caulimoviruses from different crops. At least one generic primer set proofed capable of detection of all caulimovirus tested to date. Sequence analysis of the PCR products enabled subsequent identification of the individual viruses. Use of these primer sets will allow NPPOs and inspection services to more reliable monitor caulimovirus infections and spread.

## PP-3_81: Induction of systemic resistance in *Lycopersicon esculentum cv.*PKM1 against *Cucumber mosaic virus* by using plant growth promoting rhizobacteria (PGPR)

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Studies were done to evaluate two specific strains of plant growth promoting rhizobacteria (PGPR) for induced systemic resistance (ISR) against *Cucumber mosaic virus* (CMV) in *Lycopersicon esculentum cv.*PKM1 (tomato), based on superior performance in the green house. In the greenhouse trials, PGPR treated plants were challenged by mechanical inoculation with CMV and the percentage of infection was scored based on ELISA results. For the initial screening experiment, ten of the best PGPR strains were selected for evaluation. The range in the percentage of plants exhibiting CMV symptoms after 10 days of inoculation was 50-60% compared to 100% of plants with symptoms in the control plants inoculated with CMV, but not treated with PGPR. In the second series of green house experiments among the ten PGPR strains, treatment with four PGPR strains resulted in 30- 35% of symptoms, compared to 92% of control plants with symptoms. Finally, in the third set of green house experiments, one among the four PGPR strains was chosen based on the efficiency for further evaluation in the field experiments. The isolated bacteria was identified as *Bacillus subtilis* and the isolate was named as *B.S 3A25*. The percentage of plants with CMV symptoms in the *B.S 3A25* treatment was 17%, compared with control plants showing 81.5% of symptoms. These data showed that treatment of tomato plants with *B.S 3A25* strain resulted in significant enhancement of growth and protection against infection by CMV.

## PP-3_82: Production of polyclonal antiserum against the RTBV CP gene expressed in *E. coli* for the detection of *Rice tungro bacilliform virus*

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Use of ELISA for tungro viruses is still very limited in rice-growing countries because of difficulty in purification of tungro viruses which is present low titre, its confinement to vascular tissues and the difficulty of separating two viruses biologically. Here we have reported polyclonal antiserum production by using recombinant viral coat protein expressed in *E. coli*. The clone of RTBV CP gene expressed in *E. coli* was multiplied in LB broth and the liquid culture was induced with IPTG for the induction of coat protein as a fusion protein along with bacterial beta-galactosidase. The induced protein band of approximately 157 KDa was separated from the SDS-PAGE and the protein was eluted by dialysis. The protein concentration was estimated and 0.90 mg/ml of antigen was injected intramuscularly in rabbit and the serum was collected 15 days after last injection. The titre value of the developed antiserum of the RTBV was determined by using indirect ELISA. The antigen and antibody were tested at different dilutions. The titre value of the antiserum raised against the coat protein expressed in *E. coli* was found as 1: 5000. The tungro infected samples maintained at Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore-3 were tested using the antiserum developed against CP gene expressed in *E. coli*. Results revealed that antiserum developed in this study successfully detected the presence of RTBV in the tungro infected plant samples. At 1:5000 dilution of antiserum, the sample collected from PBS, Coimbatore (positive check) recorded high concentration of virus (1.141) followed by other leaf samples *viz.,* Kanyakumari1 (0.949), Kanyakumari 2 (0.942), Kanyakumari 3 (0.879) and viruliferous leafhopper (0.827).

## PP-3_83: Serodiagnosis of mixed infection of *Ilar* and *Tospo* viruses in mungbean in Tamil Nadu, India

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Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important pulse crop in India, Southeast Asia, Africa, South America and Australia next to Urdbean (*Vigna mungo* L. Hepper). As such a number of virus diseases cause huge damage to the crop which results in loss of revenue to the farmers and country. Of late stem necrosis caused by *Tobacco streak Ilarvirus* (TSV) and leaf curl disease caused by *Peanut bud necrosis Tospovirus* (PBNV) have became a serious threat to the cultivation of this crop. TSV infection on mungbean under field condition was characterized by brown necrotic areas in the leaves, petiole, necrosis of stem and drying of the plants from the tip. On the other hand PBNV infection resulted in necrosis/chlorosis of vein, midrib, petiole, stem, growing point, bud and pod. When these viruses viz., TSV and PBNV appears in tandom reduction in internodal length and axillary shoots with severe stunting was very severe. However, on the young leaves, vein necrosis along with chlorotic spots and on the matured trifoliate leaf necrotic streaks, reduced lamina area, vein necrosis, yellowing on the leaf margin and chlorotic spots were also observed. The nucleocapsid (N) protein of mungbean *Ilarvirus* and *Tospovirus* isolate was purified and polyclonal antiserum was developed separately for TSV and PBNV and titre value has been fixed by adopting DAC-ELISA, accordingly the concentration at which the viruses can be detected were 1:1000 and 1:200 respectively. TSV antiserum could also detect *Ilarvirus* isolates from urdbean, sunflower and soybean. In addition for the first time in Kharif'2006 Bt cotton, TSV was detection with the help of the antiserum developed in this study. While, PBNV antiserum could detect PBNV isolates from urdbean and tomato. The antisera developed for these two viruses were useful to detect their respective viruses even in the samples having mixed infection of these two viruses.

## PP-3_84: Influence of temperature on the transmission of luteoviruses by apterous aphids

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Luteoviruses like *Barley yellow dwarf virus* (BYDV-PAV) in cereals and *Turnip yellows virus* (TuYV) in rapeseed are of great economic importance worldwide. These viruses are transmitted exclusively by aphids in a persistent manner. *Rhopalosiphum padi* in case of BYDV and *Myzus persicae* in case of TuYV are the main vectors in Germany. The infestation of winter cereals (barley, wheat) and winter rapeseed takes place mainly in autumn. The spread of the virus across the field depends on the temperature which has a strong influence on the multiplication rate of aphids and on the virus transmission efficiency. The relation between the increase in aphid population and temperature is well studied. But knowledge of the influence of temperature on the transmission efficiency is less comprehensive. For a better understanding of this relation the virus transmission rate by single aphids was studied under different temperatures (10, 15, 20 or 25°C) in a growth chamber. Apterous females of *R. padi* or *M. persicae* aquired the virus (isolate BYDV-PAV-ASL-1 or TuYV-BN5ASL) in different periods (1, 2 and 4 d) were transmitted on seedlings of winter barley cv. 'Rubina' or winter rapeseed cv. 'Express' (50 to 60 plants / variant) to inoculate the plants for 1, 2 or 4 days. After the inoculation the aphids were killed by insecticide spraying and the plants were cultivated in an air conditioned greenhouse (20°C, 70% relative humidity, 16 h photoperiod). Serological tests (DAS-ELISA) of the single plants were carried out 42 dpi to determine the infection rate (IR). In the case of BYDV also symptom expression was estimated by scoring the plants (score 1 = no symptoms to score 9 = plant died) and the degree of attack (DA) was calculated. The transmission efficiency of BYDV and TuYV by single aphids was clearly influenced by the temperature. The lower temperature limit for a successful transmission of both viruses was determined at 10°C. For BYDV the infection rate increased with higher temperature and longer acquisition / inoculation periods to 85.7% (25°C; 4d / 4d). The DA ranged between 0.0 (10°C; 1d / 1d) to 39.4 (25°C; 4d / 2d). The influence of the temperature on the severity of symptoms on BYDV-infected plants was low. However, there is a tendency that plants develop stronger symptoms at 20°C than at 15 or 25 °C. In the case of TuYV the infection rate increased from 2.0 % (10°C; 1d / 1d) to 85.0% (25°C; 4d / 4d). In a long-standing study of the incidence of BYDV in winter barley and winter wheat fields of Saxony-Anhalt which is located in the central part of Germany a clear correlation between the severity of virus attack in spring and the number of days with a mean temperature > 10°C in the previous autumn was found. Taking into account global warming the results give evidence to an increasing risk of growing yield losses caused by aphid-transmitted viruses in different crops.

## PP-3_85: Screening diploid banana germplasm against *Banana streak virus* by PCR and NASH

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*Banana streak virus* (BSV) disease significantly reduces productivity in some banana cultivars grown world over. In India, a popular, sturdy triploid, cultivar Poovan (AAB) belongs to Mysore sub group gets affected severely by BSV. Nearly 10 percent of banana area grown in this variety, by small and marginal resource poor farmers for their sustenance and livelihood. Though, this virus is spread by mealy bugs, the significance of the vector is negligible. The symptoms caused by BSV in Poovan are confused with *Cucumber mosaic virus* (CMV) infection. Since, BSV genome has integrated with the host genome is an impediment in breeding and movement of banana germplasm. In this investigation, 15 BB diploid and 43 AB genomic groups, available at NRC for Banana were screened against BSV species by PCR and NASH techniques using non radio active DIG probes. Though the BSV episomal forms can be detected only by ISEM or IC-PCR, an attempt was made to find the accessions free from endogenous para-retroviral sequences (BSV). All the fifty eight accessions used in the study did not show any external symptoms, characteristic of the streak disease. Total DNA was isolated from all the accessions using a modified CTAB method. The primers used were specific to BSV-Mys, IM, RD, GF as described by Geering *et al* (2000). The episomal, mixed primers reported by Dallot *et al* (2001) and a primer was designed based on the published BSV-OL strain sequence were also used. BSV-Mys and BSV-OL specific non-radioactive DIG labeled probes prepared using PCR probe synthesizing kit (Roche Diagnostics) were used for detection. Among 15 BB diploid accessions screened, Athiakol 1 (0011) was negative for all the seven sets of primers tried which include a mixed primer to detect the integrant sequence, where as *M. balbisiana* (0508) was positive for all the primers tested. BSV-GF specific primer was amplified only by *M. balbisiana.* Episomal primer derived from BSV-OL was amplified by 12 BB accessions. Bhimkol 2 was positive for BSV-Mys and BSV-RD primers, similarly Attikol was positive for episomal and BSV-Mys primers. It is interesting to note that many BSV species specific sequences are found in the BB clones of India and these positive amplifications might be from BSV-EPRV's. As there are variations among BB clones with respect to BSV EPRV's, it is suspected that the pattern of integration and copy number might vary among the accessions. Among 43 accessions of AB diploids tested with PCR and NASH, 25 were positive for BSV-OL specific probe and only 20 were positive for BSV-Mys specific probe. Narmine was highly positive for both probes. 13 accessions were free for both the probes tested. By the PCR test, 29 accessions were positive for episomal primers and 18 were positive for RSR primer. Accessions positive for episomal primers were also positive with RSR primer except in one accession. Implications of this result in banana breeding and improvement in India are discussed in detail.

## PP-3_86: Effect of plant extracts and derivatives, butter milk and virus inhibitory chemicals on watermelon mosaic virus infection in cucumber

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Watermelon mosaic disease is a common virus disease on watermelons (*Cucumber sativus* L.) in Eastern Uttar Pradesh, India. Disease symptoms consist of vein clearing, mosaic mottling, shortening of internodes and deformed fruits. In a glasshouse experiment conducted during summer season of 2006, leaf extracts from 10 plant species (*Boerhaavia diffusa, Baugainvillea spectabilis, Croton bonplandianum, Ipomea carnea, Mirabilis jalapa, Oscimum basilicum, Prosopis chilensis, Vitex negundo, Catharanthus roseus, Azadirachta indica*) were evaluated in three replicates for their efficacy against virus infection responsible for mosaic disease. Fresh green leaves were collected from fully mature plants and extracts were prepared by homogenizing leaf tissue in water at 1g/10 ml. The homogenate was filtered through two folds of muslin cloth and 10% (v/v in water) leaf extract was sprayed on the primary leaves of *C. sativus* var. Vinayak. After 24 h of leaf extract application test plants were inoculated mechanically with sap extracts prepared from the mosaic disease-affected plants. Test plants sprayed with *Baugainvillea spectabilis* and *Boerhaavia diffusa* were least infected with disease (93.3% and 91.7%, reduction in incidence, respectively). Whereas only 43.3% of plants sprayed with *Vitex negundo* were infected. Spraying of neem oil, Thuja-30 and buttermilk were also effective in reducing the disease incidence. However, there was no significant reduction in disease on plants sprayed with acetyl salicylic acid and barium chloride. Control plants (untreated) were 100% infected in this experiment. It appears that various treatments used are either blocking virus entry into plants or inducing resistance response to counter the virus infection. Further studies are needed to understand the mechanism.

## PP-3_87: Management of tomato leaf curl virus disease and its whitefly vector on tomato (*Lycopersicon esculentum* Mill) in eastern Uttar Pradesh, India

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Tomato leaf curl virus disease (TLCVD), transmitted by whitefly, *Bemisia tabaci* Gen. in a persistent manner, is the most economically important disease of tomato in India. In this study efficacy of seven plant product viz., *Clerodendrum aculeatum* (10%,w/v),*Mirabilis jalapa* (10%,w/v), *Sorghum vulgare* (10%,w/v), *Bougainvillea spectabilis* (10%,w/v), *Ocimum sanctum*(10%,w/v), *Prosopis chilensis* (10%,w/v) and Neem (*Azadirachta indica*) seed kernal (5%,w/v) and two insecticides, endosulfan (0.05%,w/v), monocrotophos (0.05%,w/v), were evaluated for the management of TLCVD and *B. tabaci*. Two separate field experiments were conducted in randomized block design with 3 replications in 3m x 2m during summer of 2005 at Balrampur, Uttar Pradesh, India. A popular tomato cv. Pusa Ruby was sown in the field at 45 cm x 30 cm spacing between row and plants. Each extract was applied at 250 l/ha at 2 week intervals. Spraying with neem seed kernel extract, and leaf extract of *C. aculeatum* and *M. jalapa* proved most effective in reducing the number of whiteflies and consequently reducing the disease pressure and increased yield. Up to 60% reduction in disease incidence was observed in trials sprayed with *C. aculeatum* leaf extracts, as against 70% TLCVD infection in controls. Maximum fresh fruit yield (3.66 t/ha) was obtained from the plots treated from *C. aculeatum* as against 2.26 t/ha in untreated control plots. The present study reports the comparative efficacy of various natural plant products and insecticides used against incidence of TLCV disease and whitefly population under field conditions.

## PP-3_88: Detection and management of viral disease complex of soybean

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Studies were conducted to identify the viruses associated with the chlorotic spots, bud and stem necrosis and yellow mosaic symptoms in the soybean experimental farms of Tamil Nadu Agricultural University, and it was identified as *Tospovirus, Ilarvirus*, and *Geminivirus* isolates associated individually and in combinations. Purified preparation of the *Tospovirus* and *Ilarvirus* isolates consisted of a major polypeptide of approximately 30 and 29 kDa respectively in SDS- PAGE analysis. Polyclonal antiserum was developed against *Tospovirus* and *Ilarvirus.* Serological studies revealed that soybean *Tospovirus* isolate reacted positively with *Peanut bud necrosis virus* (PBNV) and *Watermelon silver mottle virus* (WSMV) antisera and negatively with *Tomato spotted wilt virus* (TSWV). Soybean *Ilarvirus* isolate reacted positively with antiserum of *TSV* isolates of soybean and sunflower. Soybean *Geminivirus* isolate reacted positively with *African cassava mosaic virus* (ACMV) polyclonal antibody and *Indian cassava mosaic virus* (ICMV) monoclonal antibody (SCR 18).The coat protein gene of soybean *TSV* isolate under study shared more than 99 per cent identity with the *Tobacco streak virus* of urdbean. The coat protein gene of soybean *Geminivirus* isolate shared 40.4-43.0 identity with the *mungbean yellow mosaic virus.* The biological and serological studies of soybean *Tospovirus* isolate were similar to *PBNV* and it is proposed that the soybean *Tospovirus* should be regarded as a strain of *PBNV* belonging to *Watermelon silver mottle virus* (*WSMV*) serogroup. Foliar spraying of thiamethoxam (0.2g/l), seed treatment of Imidacloprid (5g/kg) and *Clerodendron inerme* (10 %) at fortnightly intervals were found effective in the management of soybean viruses under field conditions.

## PP-3_89: Identification of *Cotton leaf curl Rajasthan virus* infecting tomato in Pakistan

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Tomato leaf curl disease (ToLCD) is the major limiting factor of tomato production in Pakistan. The disease is caused by whitefly-transmitted geminiviruses (genus *Begomovirus*, family *Geminiviridae*) and occurs in all the tropical, subtropical and, increasingly, temperate regions of the World. In India and Pakistan the disease is caused by a bipartite begomovirus, *Tomato leaf curl New Delhi virus* (ToLCNDV), or one of a number of monopartite begomoviruses, the majority of which associate with a recently identified symptom modulating satellite molecule termed DNA β. Three tomato samples showing phenotypically distinct symptoms of tomato leaf curl disease (ToLCD) were collected in Faisalabad, Pakistan. These exhibited a severe downward leaf curling with enations on the lower side of the leaf (plant A), a bunchy appearance of new growth with extremely small deformed leaves (plant B) and a generalized chlorosis with mild leaf curling (plant C). In order to identify the begomovirus components associated with the three disease, DNA extracted from them was screened by PCR using specific primers for ToLCNDV DNA A and DNA B. Additionally the samples were screened with a universal DNA β primer pair (designed to detect all DNA β molecules). Sample A was found positive for ToLCNDV DNA A and DNA β. Sample B was positive for DNA A, DNA B and DNA β, whereas sample C was positive for DNA A and DNA B only. The fragments amplified from plant A were cloned and sequenced. The begomovirus sequence obtained showed the highest levels of sequence identity (99%) to *Cotton leaf curl Rajasthan virus* (CLCuRV), a virus previously identified in cotton showing symptoms of cotton leaf curl disease (CLCuD). The sequence of DNA β showed 99% identity to the DNA β associated with CLCuD. This is the first time CLCuRV has been identified in tomato and indicates that this host can serve as a reservoir for the agent causing CLCuD. Partial repeat constructs for *Agrobacterium*-mediated inoculation are being produced to show infectivity of these clones (to fulfill Koch's postulates), study their host range and potential threat to crops.

## PP-3_90: *Ageratum enation virus* causes yellow vein disease of *Sonchus oleraceus*

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Weeds species are believed to act as a reservoir hosts for many economically important plant viral diseases. In a survey, leaf samples of *Sonchus oleraceus* (*Asteraceae*) plants showing yellow vein symptoms were collected from the fields of University of the Punjab, New campus, Lahore. Full-length clones of a begomovirus and a DNA β satellite were isolated from these samples. Sequence analysis showed these to be most closely related to *Ageratum enation virus* (AEV) and *Ageratum* leaf curl disease DNA β, respectively. This is the first report of AEV in Pakistan and the first sequence of a DNA β associated with the virus. The clones were shown to be infectious to plants inducing typical disease symptoms. AEV is closely related to a number of viruses causing cotton leaf curl disease (CLCuD; a major constraint to cotton production in Pakistan). We conclude that yellow vein disease of *S. oleraceus* is caused by AEV in conjunction with a DNA β satellite and that this virus is a potential threat to cotton in Pakistan, should the virus associate with CLCuD DNA β.

## PP-3_91: Occurrence of cucurbit viruses in Punjab, India

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More than 35 viruses have been isolated from cucurbits. The main cucurbit crops grown in Punjab are muskmelon, watermelon, cucumber, summer squash, pumpkin, bitter gourd and sponge gourd. Virus causes severe diseases in these cucurbits showing various kinds of symptoms. This study was undertaken to assess the occurrence of viruses in cucurbits in Punjab more specifically the symptoms variability and viruses associated with disease losses. During a 2-year study of diagnosis of virus diseases of cucurbits in Punjab state, we found that different viruses were associated with severe disease symptoms depending on the year and stage of crops. In 2006, *Cucumber mosaic virus* (CMV) caused the most severe disease in muskmelon, squash and snap melon at early stage of crop by producing blistering kind of symptoms. In year 2007,due to frequent rainfall at early stage of crop aphid vector population could not buildup and no CMV incidence was observed but at maturity four new viruses were detected for the first time in Punjab and caused severe losses in muskmelon, snap melon, squash and other cucurbits. Enzyme-linked immunosorbent assay (ELISA) of field samples showed association of *Zucchini yellow mosaic virus* (ZYMV) with yellowing type of symptoms, whereas, brittleness, clustering and reduction in size of apical leaves were associated with *cucumber green mottle mosaic virus* (CGMMV), mosaic were associated with *squash mosaic virus* (SqMV). Watermelon mosaic strain of *papaya ring spot virus* (PRSV) was also detected. Virus associated with bitter gourd and muskmelon showing patchy chlorosis and vein bending type symptoms could not be detected. Similarly pumpkin showing yellow vein type symptoms did not react with any of these antisera. In field samples infected with mixture of CMV, ZYMV, CGMMV, SqMV and PRSV viruses, CMV usually predominated after rub inoculation of susceptible test plants.

## PP-3_92: Development of coat protein gene mediated resistance to *Tobacco streak virus* in groundnut

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Fertile transgenic lines of peanut/groundnut (*Arachis hypogaea* L) cultivar JL-24 ($T_0$) against *Tobacco streak virus* (TSV) were generated using coat protein (CP) gene in sense and antisense orientation by *Agrobacterium*- mediated transformation of de-embryonated cotyledons. About six-seven months were required between explant transformation and hardening of transformants in the glasshouse. Southern analysis revealed successful integration of the CP gene in two transgenic lines (S15, AS2) with single copy and one transgenic line (S3) with two copies. Northern analysis also indicated the presence of CP transcript in transgenic lines with CP gene in sense as well as antisense orientation. CP expression was observed in transgenic lines with sense CP gene (S3, S15) by DAC-ELISA. Further analysis of transgenic lines ($T_1$) revealed successful transfer of the gene to the next generation.

## PP-3_93: Molecular detection, DNA-1 based sequence identities and phylogenetic analysis of five Indian isolates of *Banana bunchy top virus*

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Banana bunchy top disease (BBTD) caused by *Banana bunchy top virus* (BBTV) is the most serious threat of banana cultivation worldwide. The disease was first recognized in Fiji in 1889 then introduced in Sri Lanka in 1913 and later transmitted to Southern India in 1940 from where the virus spread to the various banana-growing areas of India. BBTV was isolated from infected banana in Australia. Further BBTV isolates were investigated from Fiji, Egypt, Vietnam, India, Japan, Philippines, China and Pakistan. We conducted a survey in banana-cultivating areas of Lucknow, Barabanki, Bahraich, Kanpur and Etawah districts of Uttar Pradesh in India and infected samples of banana (*Musa paradisiaca*) cv. Harichhal were collected. To detect BBTV infection, the total DNA was extracted from naturally infected plant samples and PCR was performed using specific primers of BBTV DNA-1. PCR resulted positive amplification of ~1.1 kb in all the infected samples of five different locations. To confirm the authenticity of PCR amplicons, the bands from gel was transferred on to the nylon membrane and allowed to hybridize with a homologous probe prepared from the clone of BBTV partial Rep gene (GenBank accession no. DQ104700). PCR amplicons were cloned, sequenced and sequence data obtained were submitted in the GenBank database under the accession no. DQ256267, DQ285570, DQ285571, DQ656118 and DQ656119. The analysis of sequence data revealed presence of complete sequence (1,111 nt) of DNA-1 in all five BBTV isolates, which contained two ORFs: the major gene encodes replicase protein of 33.6 KDa (which contains the nucleotide-binding motif, GGEGKT) and an internal ORF encodes a putative protein of 5kDa of unknown function. Sequence data of these isolates were aligned and compared with other isolates of BBTV reported from India and abroad which showed maximum 94-98% identities with isolates of South Pacific group and 82-87% (>90%) identities with the isolates of Asian group. Phylogenetic analysis of nucleotide sequence of DNA-1, replicase gene and internal ORF showed its close relationships with isolates of South Pacific group rather than Asian group. Therefore, the isolates of BBTV under study were identified as the members of South Pacific group.

## PP-3_94: Detection of phytoplasma in ornamental and economically important plants

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Phytoplasmas are mollicutes belong to the monophyletic order *Acholeplasmatales*. Phytoplasma are characterised by their lack of a cell wall, a pleiomorphic shape, normally with a diameter less than 1micrometer, and their very small genomes. Phytoplasmas are pathogens of important crops, causing a wide variety of symptoms. They are most prevalent in tropical and sub-tropical regions of the world. Phytoplasmas require a vector to be transmitted from plant to plant and this normally takes the form of sap sucking insects such as leaf hoppers in which they are also able to replicate. Witches broom, little leaf, yellows and phyllody symptoms were noticed on Chrysanthemum, Adenium, Marigold, Catheranthus, Pigeon pea, Sesame, Brinjal, Parthinium, Phylanthus, Linseed plant species rowing in various place of Uttar Pradesh, India with a significant disease incidence. The total nucleic acid isolated from infected leaf samples. The DNA samples were blotted on nylon membrane and allowed to hybridise with a probe prepared from the clone of chilli little leaf phytoplasma reported earlier (GenBank Accession no. DQ343288). Most of symptomatic plants showed strong hybridization signals, indicating an association of the phytoplasma. PCR of was performed with P1/P6 universal primers specific to the 16S rRNA gene (Deng & Hiruki, 1991) using the total DNA isolated from Chrysanthemum, Adenium, Pigeon pea and Sesame. In addition, nested PCR was carried out with primers R16F2n/R16R2n (Gundersen & Lee, 1996) using the first round PCR product as the template. DNA fragments of the expected sizes (~ 1.5 kb and 1.2 kb respectively) were obtained from the infected plant samples. The 1.2 kb amplicons were cloned, sequenced, and the sequence data deposited in GenBank (Accession No.DQ431842, EF159729, DQ343287 and DQ431843). Blast analysis of Chrysanthimum (DQ431842) and Adenium (EF159729) isolates showed 99% sequence similarity with the 16S rRNA gene of Ash witches'-broom, Maize bushy stunt, Dogfennel yellows, *Epilobium* phyllody, Onion yellows and Aster yellows phytoplasmas, belonging to the '*Candidatus* Phytoplasma asteris' (16S rI) group. Pigeon pea (DQ343287) and Sesamum (DQ431843) isolates showed the highest (99%) similarities with Ash witches'-broom, *Hydrangea* phyllody, Maize bushy stunt, *Epilobium* phyllody, Onion yellows, Barley deformation, Aster yellows and Silene virescence, members of '*Candidatus* phytoplasma asteris' (16SrI) group. Phylogenetic analysis of these isolates also showed a close relationship with the members of *Candidatus* phytoplasma asteris' (16SrI) group

## PP-3_95: Correlation of environmental factors in relation to PVX and PVY disease severity and aphid population

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Twenty nine test cultivars/ lines were planted in the research area of Department of Plant Pathology University of Agriculture Faisalabad to study the relationship of environmental factor with disease severity of PVX and PVY and aphid population. Disease severity of PVX and PVY and aphid population was recorded on weekly basis and subjected to correlation analysis with different environmental factors having significant influence on disease severity. The overall correlation of maximum (22-25$^0$C) and minimum (5-7$^0$C) air temperature and pan evaporation (2.0-3.6 mm) was highly significant but negative with disease severity while positive with relative humidity (45-70%) and aphid population. Aphids carry virus from a central source to adjacent healthy plants, and these plants must become systemically infected before they can serve as sources of virus for further spread which was correlated with PVY. Thus relative humidity and aphid population seems to play crucial role, especially aphid population in the spread of PVY. If distance traversed by aphids from the central virus source plant is distributed normally, many aphids reach plants nearest to the central source but few reach maximum distances. Thus, the nearest plants are exposed to larger numbers of aphids from the virus source than more distant plants. They are also the first exposed after aphids acquire virus when transmission efficiency of aphids is highest. They are exposed at a younger, more susceptible age, especially aphid in the spread of disease.

## PP-3_96: Recent advances in breeding for resistance to viruses in legumes: lessons for pigeonpea

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Breeding of legumes for resistance to viruses has been considerably improved today by developments in molecular biology and biotechnology. Several naturally occurring virus resistance genes have been reported and characterized in legumes and their wild relatives. Techniques have also been developed that modify the virus genomes, detect specific gene products and their functions, transform plants that elicit novel forms of resistance to viral diseases, and elucidate gene silencing and recombination. However, such progress has been limited to only a few legumes such as beans (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and the model legumes (*Medicago truncatula* and *Lotus japonicus*). With the increasing effort to ensure food security for the world's poor population, there is a need to widen and expand research to other neglected but equally important legumes such as pigeonpea (*Cajanus cajan* L.). The subject of comparative genomics has recently received much focus for its ability to enable the transfer of knowledge between models and organisms. This study discusses specific characterized virus resistance genes that have had an impact in legume improvement including their genetic context, genomic organization, mechanisms of resistance and agricultural success. The study further discusses how comparative genomics would facilitate the transfer of this knowledge to the genome of pigeonpea.

## PP-3_97: Persistence and infectivity of an Iranian isolate of *Tomato yellow leaf curl virus* in whitefly (*Bemesia tabaci*)

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*Tomato yellow leaf curl virus* (TYLCV) is a major threat to tomato production in the tropical and subtropical regions as well as in the greenhouses of the temperate zone. Development of TYLCV epidemics in the recent years was largely affected by the changes in the interaction of virus strain, vector population and the host genotype. Persistence and infectivity of a newly reported TYLCV isolate, TYLCV-Ir2, from Iran were studied. Virus free whiteflies reared on cauliflower plants were fed on the TYLCV infected tomato plants for 48 hours and were then put back on the none host cauliflower plants to examine the persistence of the virus in its insect vector. TYLCV infected whiteflies were tested for the presence of viral DNA using PCR and for its infectivity by testing their ability to infect healthy tomato plants. A 670 bp fragment of viral DNA was detected from whiteflies until $12^{th}$ day of living on cauliflower plants, while their ability to infect tomato plants was just four days as tested after three, four and seven weeks post inoculation. These observations were further confirmed by back inoculation tests. Results of this study suggested that although viral DNA can persist in the life span of an infected adult whitefly, however, the infectivity can be retained only less than 6 days post acquisition.

## PP-3_98: Production of monoclonal antibodies against *Cowpea mottle virus*

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Cowpea (*Vigna unguiculata*) is an important source of protein in tropical countries especially in regions where animal proteins are scarce. Production of this crop is limited by pests and diseases. *Cowpea mottle virus* (CMeV; genus *Carmovirus*) is one of the economically important cowpea infecting viruses prevalent in the Nigeria, causing yield loss as high as 75%. This virus is seed-borne in cowpea (10%) and also has coleopteran vectors (*Ootheca mutabilis, Paraluperodes lineata*). This makes the risk of virus spread through germplasm materials exchanged between regions and further spread within the fields by vectors. The need for a reliable and sensitive diagnostics technique for the detection of this virus is important for its management and hence the reason for this piece of work. CMeV (250 μg/0.5 ml) purified from experimentally inoculated cowpea (cv. Ife brown) plants was emulsified in 1:1 (v/v) Freund's complete adjuvant and used for immunizing 3-weeks old Balb/c mice by subcutaneous injection. Another two injections of purified virus (emulsified in Freund's incomplete adjuvant) were given at weekly intervals and a booster dose was give a month after the third injection. A month after the final injection, mice spleen and thymus were harvested and used for fusion with myeloma cells lines using PEG-based fusogen and cells were diluted in Dulbecco's Modified Eagles's Media supplemented with 20% fetal calf serum and hypoxathin, aminopterin, thymidine (DMEM-HAT) and they were plated out at 100 µl per well Screening for the antibody producing cells was done from 9$^{th}$ day after the fusion. Direct antigen coating (DAC) or triple antibody sandwich (TAS; using CMeV polyclonal antibodies for virus trapping) enzyme-linked immunosorbent assay (ELISA) with alkalinephosphatase detection system was used for screening the clones. Hybridoma cells were found in 492 (85%) wells. Screening for antibodies has identified anti-CMeV immunoglobulin-producing cells in only one well. After expansion and sub-cloning of the single cell lines, four cell lines, 5B4, 5C5, 5C8 and 5E10, that produced CMeV specific antibodies were selected. The IgG extracted from four monoclonal cell lines had a titer of 0.1 μg/ml in DAC-ELISA. When used at 1 μg/ml monoclonal antibodies detected CMeV antigen 1:3000 (w/v) infected cowpea leaf tissue extracts. The absorbance values at 405 nm for the 0.5  g/ml concentration of IgG from the four monoclonal cell lines for the detection of CMeV in the infected cowpea leaves (1:10 w/v) were 0.592 (±0.033), 0.747 (±0.088), 0.963 (±0.084) and 1.205 (±0.033) for 5C8, 5C5, 5E10 and 5B4, respectively. This suggests that antibodies produced from 5B4 cell line as most sensitive. However, IgG isolated from the four monoclonal cell lines did not react with denatured CMeV coat protein in Western-immuno blot assay. The monoclonal antibodies developed are suitable for sensitive and reliable detection of CMeV by ELISA.

# Poster Session – IV

## PP-4_99: Gene silencing suppressor ability of *Tomato leaf curl virus* AC2

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The major symptoms produced by *Tomato leaf curl virus* (ToLCV) are leaf curl and yellow mottle. Leaf curl is produced by a monopartite ToLCV virus (DNA A) and mottle is produced by bipartite virus (DNA A and DNA B). Both types have satellite DNA β associated with them. AC2 is a transactivator of geminivirus transcription and is also a suppressor of gene silencing. A comparison of the sequences of the AC2 gene from ToLCV isolate producing leaf curl and the mottle types and the gene silencing suppression ability of the encoded proteins will be presented.

## PP-4_100: Non-translatable coat protein of PRSV-P confers resistance in transgenic papaya cultivar Solo

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*Papaya ring spot virus* (PRSV), belonging to the family *Potyviridae* and transmitted by many species of aphids, is presently the most widespread and damaging viral disease of papaya, severely affecting its cultivation in India. Genetically engineered papaya resistant to PRSV were developed and commercialized for the first time in Hawaii. However, these transgenic lines resist only the Hawaiian isolates of PRSV-P and not isolates from other countries. Hence, there is a need to develop transgenic papaya that are adapted to Indian conditions and which resist local strains of the virus. We have cloned the coat protein gene of the Bangalore isolate of PRSV-P and used it to develop a marker-free non-translatable plant expression construct. This construct was used in direct DNA uptake method of transformation, and the validity of the construct was confirmed through studies on transient expression of RNA in leaf disks of papaya. Plants of papaya cultivar Solo were transformed by *in-vivo* electroporation of 2 month old seedlings. Chimeric $T_0$ plants were screened by PCR to check for the integration of the transgene. $T_1$ transgenic plants were raised by selfing the T0 plants. One hundred and twenty five seedlings were raised and were screened by PCR. A total of 45 primary transformants have been obtained which harbour the complete cassette. These primary transformants have been challenged twice at the seedling stage, both mechanically with purified PRSV virions and through aphid vector. Twenty-two plants are immune to the disease 2 months after the challenge. Sequencing of the coat protein clone as well as the insert in two transgenic plants revealed that the potential transcript is non-translatable due to the presence of several stop codons. Southern blot analysis showed integration of a single copy in two of these transformants.

## PP-4_101: Cloning and sequencing of complete RNA 3 genome for molecular identification of *Cucumber mosaic virus* causing shoestring on tomato in India

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Tomato is an important vegetable crop cultivated over 5 lakh hectares with the total annual production of about 7600 metric tones in India. Several viruses like *Cucumber mosaic virus, Tomato leaf curl virus, Tomato bushy stunt virus, Tomato spotted wilt virus* and *Tobacco mosaic virus* infect tomato cultivation and cause great economic losses to the country. *Cucumber mosaic virus* (CMV) is the major virus responsible for severe losses in the commercial production of tomato. In nature CMV is usually introduced to tomato crop by aphids in non-persistent manner from wild reservoir hosts (weeds). Severe mosaic and shoestring disease with significant disease incidence was observed during 2004-05 in various cultivation areas of Uttar Pradesh. The virus was successfully transmitted mechanically and through aphid (*Myzus persicae*). Leaf dip preparation revealed the presence of 28 nm spherical core particles under electron microscope, the characteristic feature of cucumovirus. In ODDT crude antigen reacted strongly with CMV antiserum (PVAS 242a, ATCC, USA). Mechanical transmission (sap and aphids) of the virus, serological tests and electron microscopic studies evidenced the presence of CMV associated with shoestring symptoms on tomato; therefore, the virus isolate was further characterized at molecular level on complete RNA3 sequence analysis. To amplify RNA3, total RNA was isolated from 100 mg young leaf tissue of naturally infected tomato plants and RT-PCR was performed using total RNA as a template and a pair of primers specific for RNA 3 genome. Amplicon was cloned and sequenced. Sequences data was analyzed by the Entrez program using BLAST and submitted to GenBank (EF153734). The complete RNA3 sequence analysis of the isolate under study showed maximum 98% nucleotide sequence identity with the Tfn and Nt9 isolates of CMV reported from Italy and Taiwan respectively. Coat protein and movement protein gene also showed highest 98% sequence identity with the Nt9 and Tfn isolates which support the analysis carried out at complete RNA 3 level. In phylogenetic analysis Ts, Tfn and Nt9 isolates grouped in same cluster, indicating a common ancestor. On the contrary, Indian CMV isolate of banana, amaranths, datura, rauvolfia, jatropha and chrysanthemum showed only 92% nucleotide identity with Ts isolate and clade in different cluster in phylogenetic tree indicating the divergent relationship of isolate under study with the previously reported isolates of CMV from India.

## PP-4_102: Research on BYDV in Uzbekistan

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*Barley yellow dwarf virus* (BYDV) widely spread in the world. It have been noted all continents, where cultivated cereal crops. BYDV was first identified in Uzbekistan in 2001. BYDV-PAV, -MAV and SGV, also *Cereal Yellow dwarf virus* (CYDV-RPV) were revealed and described. We monitored BYDV on wheat and barley crops in the Tashkent, Jizzah, Samarkand and Kashkadarya regions during 2001- 05 and study distribution of virus. We observed and compared irrigated and boghara regions. Results of monitoring showed, that BYDV was wide spread on the wheat fields in irrigated condition than boghara. Especially, on fields where wheat is cultivated continuously throughout the year. BYDV is transmitted 5 species of aphids that attack wheat in Uzbekistan: these are *Sitobean avenae* L., *Rhopalosiphum maidis* Fitch., *Schizaphis graminium* Rond, *Diuraphes noxia* Mordv, *Metopolophiumdirhodum* Walk. However, BYDV–PAV and its vector *Sitobean avanae* L distributed widely and BYDV–PAV is the dominant isolate then other isolates. BYDV was found to infect cereal grasses, Polypogon Desf., Cynodnon Rich, Sorghum halepense (L) Pers, Pao L., Echinocloa crus-galli (L) et. Sch., that grows around fields and in the garden. They play important role as reservoirs of BYDV in the nature. *Phragmites communis* Trin., is immune to BYDV, but maize was susceptible. The results of our investigations showed, that BYDV infects several cereal grasses and maize in summer, then to winter wheat and barley crops in autumn and also in winter and spring. To identify BYDV resistant wheat varieties, we artificially infected local varieties and ICARDA's winter wheat, triticale and barley lines with BYDV-PAV and concluded that all local varieties (Marjon, Polvchanka, Demetra, Intensiv and others) were susceptible to the infection and ICARDA's lines were resistant.

## PP-4_103: *Pepino mosaic virus*: variability in strains

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*Pepino mosaic virus* is an example of a plant virus that has caused significant agronomical problems in a relatively short period of time. Since its first description from Pepino plants (*Solanum muricatum*) collected in 1974 in Peru, the virus remained insignificant for a long time. A tomato strain manifested itself in commercial tomato crops in Europe in 1998. Since then the virus has been reported worldwide and has become an important virus disease in commercial tomato production. Recently a number of new strains of the virus have been described. These strains differ significantly in sequence from the tomato and Pepino strains. These new strains may play an important role in the increase of the agricultural importance of PepMV. Not only between but also within strains, sequence differences and variations are observed. These range from minor point mutations to recombinations between strain specific sequences. With the growing spread of molecular detection and diagnostic techniques, often relying on the presence of specific sequences, these sequence variations will inevitably lead to false negative results. These may have serious economic consequences. Sequence variations between different PepMV isolates were studied and used to design general PepMV primers as well as strain specific primer sets. These primer sets may play an important role in reliable detection programmes as well as epidemiological studies on the distribution and spread of the virus and its strains.

## PP-4_104: Molecular variability in the non-structural (NSs) gene of the *Peanut bud necrosis virus* Isolates from India

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Four of the sixteen tospoviruses reported in India, *Peanut bud necrosis virus* (PBNV) is increasingly becoming major constraints to the production of quality vegetables in different regions of the country. During a survey in 2006, field collected tomato and chilli plant samples exhibited characteristic symptoms induced by tospovirus from different regions of Karnataka and Andhra Pradesh States, India. The symptoms under field conditions include chlorotic/necrotic spots/rings on leaf, necrosis on petiole and stem and bud/floral necrosis. The representative field collected samples were being maintained on indicator host plant viz., *Vigna unguiculata* (cowpea, var. C-152) by mechanical sap inoculation. Symptomatic field collected and virus inoculated plant samples were tested positive to PBNV in direct antigen coating ELISA using polyclonal antibodies of PBNV nucleocapsid (N) protein. We found host range differences of these isolates on tomato and cowpea when transmitted by mechanical sap inoculations. N genes of all three isolates have been characterized which shared maximum identities of 97.9-98.6 and 98.9-100% identities at nucleotide and amino acid level respectively. Using gene specific primers, the complete non-structural (NSs) gene (1320bp) encoded by S RNA was amplified by RT-PCR. The amplicons from three representative isolates were cloned and sequenced. The NSs gene of the three isolates is similar in size of 1320 bp and highly conserved with 97.4 and 96.8 per cent identity at nucleotide and amino acid level respectively. The host range differences among PBNV isolates and the NSs gene sequence similarities and phylogenetic relationships of PBNV with other tospovirus isolates belonging to WSoMV group will be presented.

## PP-4_105: Medicinal mushrooms - a novel source for Ribosome Inactivating Proteins (RIPs) in viral disease management

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Ribosome Inactivating Proteins (RIPs) are a family of plant proteins that exhibit several interesting biological properties. They damage eukaryotic ribosome via their rRNA *N-glycosidase* activity, which depurinates ribosomes at a specific nucleotide. There are two types of RIPs: Type 1 RIPs are single chained proteins that strongly inhibit protein synthesis in a cell free system. Type 2 RIPs are proteins consisting of two subunits (a type 1 RIP A-chain and a lectin B-chain) connected by a disulfide bond. There are several studies on mushroom bioactive proteins. The straw mushroom *Volvariella volvacea* is having several polysaccharides and fairly active oxidation enzymes. Recently, antiviral RIP volvarin was found in its fruiting bodies. It has been identified that the volvarin exhibits rRNA *N-glycosidase*, deoxyribonuclease and abortifacient activities. The shiitake mushroom, *Lentinula edodes* (Berkeley) Pegler, is the most commercially important mushroom grown on wood. Shiitake is a model among functional mushrooms for extensive research of its bioactivity leading to the isolation of pure compounds which have pharmaceutical status. Medicinal properties such as anti-tumor, anticarcinogenic, anti-viral, preventive blood pressure increase in hypertension cases, and hypocholesterolemic have been attributed to active substances extracted from *L. edodes*. Antibacterial, antifungal and antiviral activities were also reported. The antimicrobial activity of *L. edodes* against *Trichoderma*, the main genus detected in damaged bed log. Antiviral proteins from medicinal mushrooms can also be used for the development of virus-resistant crop plants. These RIPs can thus be explored as a very potential candidate for biotechnological manipulations for developing, transgenic plants with virus resistant. However, a better understanding of the resistance mechanism is a prerequisite for realizing the potentials of these Rips. Hence, we are proceeding towards the isolation and characterization of RIPs (Volvarin) from the young sporocarps of paddy straw mushroom.

## PP-4_106: Cloning and sequencing of coat protein gene of *Sri Lankan Cassava mosaic virus* from Tamil Nadu, India

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Cassava mosaic disease caused by *Cassava mosaic geminivirus* is the major constraint for cassava production in India which is transmitted by whitefly *Bemisia tabaci*. *Cassava mosaic virus* infected leaves were collected from different places of Tamil Nadu, Pondicherry and Kerala. Multiplex PCR technique was employed to differentiate the causal agent of Cassava mosaic disease as *Indian cassava mosaic virus* (ICMV) and *Sri Lankan Cassava mosaic virus* (SLCMV) in the samples. Out of the twenty one samples analyzed, three samples were found to be infected with both ICMV and SLCMV. Only two samples were detected with ICMV infection which got amplified with 904 bp fragment of DNA A and all the other samples from various districts of Tamil Nadu were detected invariably with SLCMV as they amplified 599 bp of DNA A. Coat protein gene of SLCMV isolate in one of the sample collected from Salem was amplified by PCR. The amplified 800 bp fragment was cloned and the nucleotide sequence of the cloned fragment has been submitted in GenBank (accession number EF441869). The submitted nucleotide sequence was compared with other Cassava infecting geminivirus and other geminiviruses in GenBank database. Comparative sequence analysis revealed that the cloned fragment shared maximum sequence identity with *Sri Lankan Cassava mosaic virus* segment A of Salem isolate AJ607394 (65.7 %) followed by *Indian Cassava mosaic virus* (Tri) coat protein (AV1) gene AF423180 (64.6 %).

## PP-4_107: Molecular characterization of *Tobacco streak virus* (TSV) isolates of three economically important crops from Andhra Pradesh

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*Tobacco streak virus* (TSV) is wide spread across the world with more than 200 plant species recorded as being susceptible to the virus, belongs to the genus *Ilarvirus*, family *Bromoviridae*. It occurs widely in oilseeds, vegetables, ornamentals and on some weed species. The visual characteristics of this viral disease showing severe mosaic and necrosis symptoms on sunflower, gherkin and pumpkin leaves were collected from different regions of Andhra Pradesh. Leaf sap of virus suspected field samples were detected by DAC-ELISA using TSV polyclonal antiserum. The TSV positive samples in ELISA were sap inoculated onto indicator hosts like *Vigna unguiculata* cv.152, *Gomphrena globosa* and *Chenopodium amaranticolor* showed characteristic necrotic lesions on inoculated leaves. Primers were designed based on TSV RNA3 conserved sequences within the coat protein (CP) gene and 3'UTR (DVRSG3F: 5' ATG AAT AAT TTG ATC CAA RGT CCA 3' and DVRSG1R: 5' GCA TCT GGT ATA AAG GAG GCA T 3'). Total RNA was isolated from the infected leaf samples were subjected to RT-PCR, the template c-DNA was prepared by using oligonucleotide primers. Approximately 1kb PCR fragment was obtained in the RT-PCR analysis and cloned into pGEM-T Easy vector and sequenced entirely on both the strands. A 717 nucleotide length coat protein (238 amino acids) and 289 nucleotides encompassing 3' UTR region was obtained upon sequencing of the positive pGEM-T Easy clones and deposited in the GeneBank (EF159704, EF159702, EF159703). Comparative sequence analysis of the amplified product both at the nucleotide and amino acid level indicated a 97-98 % sequence homology with TSV reported on other hosts. Based on biological, serological and partial molecular studies we conclude that the virus isolated from sunflower, gherkin and pumpkin were assigned as TSV isolates from Andhra Pradesh.

## PP-4_108: Molecular cloning and sequencing of coat protein gene of *Banana bract mosaic virus* - Kerala - 1 isolate (BBrMV-KE)

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Banana (*Musa paradisiaca*) is a monocotyledonous plant belonging to the family Musaceae and one of the most important fruit crops, cultivated all over the world and is known for its nutritive value. The production and quality of banana are greatly affected by several diseases caused by various bacterial, fungal and viral pathogens. Among viruses, *Banana bunchy top virus* (BBTV), *Cucumber mosaic virus* (CMV), *Banana streak virus* (BSV) and *Banana bract mosaic virus* (BBrMV) commonly infect banana. Banana bract mosaic incidence is prevalent in many places and is spreading fast. The major problem in the management of the disease is that it remains unnoticed until it causes severe crop losses. By the time the farmer realizes the destructive nature of the disease, it often becomes extremely difficult to manage. Lack of reliable detection methods for the identification of BBrMV has led to the widespread incidence of the disease in Kerala causing enormous economic losses. The characteristic symptoms include conspicuous dark purple streaks on the inflorescence bracts, purple colored spindle streaks on petioles and pseudostem, and occasional interveinal chlorotic streaks on leaves. Total RNA from infected banana leaf (100 mg) collected from Kerala was isolated using RNA isolation kit (Qiagen) and cDNA was synthesized using primer OligodT. The partial coat protein (CP) region of this virus isolate was amplified by PCR using primers such as Bract 1 (5′ GAC ATC ACC AAA TTT GAA TGG CAC ATG G3′) and Bract 2 (5′ CCA TTA TCA CTC GAT CAA TAC CTC ACA G3′). These primers were designed to amplify a 604 bp product, including the C terminal of the coat protein and the 3′ untranslated region of the BBrMV genome, using software OLIGO 6 based on the sequence of Philippine (P1) isolate of *Banana bract mosaic virus* (BBrMV) that belongs to the genus Potyvirus, family Potyviridae. The PCR products were cloned into pGEM-T vector and analyzed. It contained an open reading frame upto 430 nucleotides and 145 amino acids and 3′ UTR comprising 174 nucleotides. The sequence comparisons of the coat protein gene using software BioEdit revealed that the Kerala isolate selected for the present study has 97 to 92.8 and 93 to 90.2 percent homology with different isolates of BBrMV (I1, CBE, TR1, TRY, BBrMV-CdM, I2, P3, BM24, I3, TH1, VT1, WS1, P2, P1, AP1) at the nucleotide and amino acid levels, respectively. It is found to be closely related to TRY, I1 and Coimbatore isolates. Details of the study will be presented.

## PP-4_109: Strainal variation of rice tungro virus disease in India

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Rice tungro virus disease (RTD) is sporadic in occurrence and its outbreak causes extensive damage to the rice crop. Though two types of virus particles, a bacilliform virus and a spherical virus are found associated in the infected tissues, the disease is thought to be primarily caused by *Rice tungro spherical virus* (RTSV). Tungro virus is transmitted in a semi-persistent manner by the green leafhoppers (*Nephotettix virescens*). The variability in virus strains is the basic survival requirement. A preliminary characterization of variability was attempted. Nine samples were collected from Chinsurah, Coimbatore, Cuttack, Kanyakumari, Puducherry, Rangareddy and Tirurkuppam. Virus infection was established on the highly susceptible TN1 plants by using vector aided transmission individually from each of the samples in isolation chambers of a glass house. The nine isolates reacted differentially on Vikramarya and Nidhi (resistant) and TN1 and expressed variable symptoms. Variation was observed in the time taken for the initial expression of symptoms and it ranged from 12 to 18 days after transmission. The symptoms varied from mild to severe stunting of the infected plants and showed yellowish to orange discolouration and interveinal chlorosis of the leaves. These results clearly demonstrated that nine RTSV isolates could be classified into three categories. Both the isolates from Coimbatore, and one isolate each from Kanyakumari and Puducherry were closely related and grouped into one category. The isolate belong to Chinsurah seem to be very distinct from all other isolates in terms of severity of the symptoms and duration of symptom expression. Total RNA was extracted from each of the RTSV isolates. Complementary DNA was synthesized using coat protein primers. Primers for regions covering coat proteins were designed using Primer 3. PCR amplification was standardized and strainal variation observed is discussed.

## PP-4_110: Rice plant growth promotion and induced systemic resistance against *Rice stripe tenuivirus* by a selected *PGPR, Bacillus amyloliquefaciens* EXTN-1

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In previous reports, treatment of *B. amyloliquefaciens* strain EXTN-1 showed a broad disease-controlling spectrum to the plant diseases caused by viral, bacterial, and fungal pathogens as well as the promotion of plant growth. In mechanisms of EXTN-1, treatment of EXTN-1 increased oxidative burst in early stage and induced the expression of resistance genes, PR-1a, PDF1.2. Mechanism involved in induced systemic resistance by EXTN-1 was revealed as simultaneous activation of salicylic acid and jasmonic acid or ethylene metabolic pathways. The purpose of this study was to determine whether *B. amyloliquefaciens* EXTN-1 has a similar effect on rice plant against *Rice stripe tenuivirus* (RSV) under greenhouse conditions. When rice seeds were soaked in *B. amyloliquefaciens* strain EXTN-1, rice plants showed significant systemic resistance against RSV as well as promoted growth. In 30-day old plants treated with *B. amyloliquefaciens* EXTN-1, the heights, weights, and lengths of roots increased by 12.6%, 9.8%, and 16.0%, respectively confirming the effects of PGPR. When the induced systemic resistance to RSV was examined, in 20-day old plants were treated with *B. amyloliquefaciens* EXTN-1, the heights, weights, and lengths of roots increased by 8.4%, 10.9%, and 4.8%, respectively compared to the control. Induced systemic resistance was more prominent in susceptible cultivars–Chucheong and Ilpum compared to the resistant cultivar, Nakdong.

## PP-4_111: Effectiveness of known resistance genes to a *rym5* resistance breaking German BaMMV strain

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The barley yellow mosaic virus complex consisting of *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV-1 and -2) causes one of the most important diseases of winter barley in North-Western Europe and East Asia. Because of its transmission by the soilborne plasmodiophorid *Polymyxa graminis* growing of resistant cultivars is the only effective method to prevent yield losses caused by these viruses. At present 16 genes conferring resistance to single strains or all members of the barley yellow mosaic virus complex are known. Resistance of the actually released cultivars in Germany (79) is due to only two resistance genes. Four cultivars resistant to BaMMV and both strains of BaYMV carry *rym5* and resistance of 50 cultivars to BaMMV and BaYMV-1 is due to *rym4*. However, investigations in France have shown that *rym5* is already overcome by a new strain of BaMMV (BaMMV-Sil). In 2004 similar observations were made in field tests for resistance carried out in Germany. Serological tests (DAS-ELISA) and electron microscopic observations detected BaMMV as the causal agent of mosaic symptoms in plants of cv. 'Tokyo' (*rym5*) from fields at Eikeloh (BaMMV-Teik) and Aschersleben (BaMMV-Tasl). Sequence analyses in the VPg coding region of the RNA1 revealed differences to the known sequence of the original BaMMV-isolate (BaMMV-ASL1, AJ 242725) and also of a French pathotype (BaMMV-Sil, AJ 544267, AJ 544268). In growth chamber tests (12 °C, 16 h photoperiod, 16 klx) the effectiveness of known resistance genes to the new BaMMV-strain was analysed. At the three-leaf-stage plants were inoculated two times at an interval of 5 to 7 days using sap of leaves of cv. ´Tokyo´ infected with BaMMV-Teik or BaMMV-Tasl. Leaves were homogenised on ice in $K_2HPO_4$ buffer (1:10; 0.1 M; pH 9.1) and silicium carbide (caborundum, mesh 400, 0.5 g/25 ml sap) was added. Five weeks after the first inoculation the number of plants with mosaic symptoms was scored and DAS-ELISA was carried out. The new BaMMV-isolates were transmitted successfully to cv. 'Tokyo' and additional cultivars carrying *rym5* ('Anastasia', 'Kamoto', 'Kyoto' 'Resistant Ym No. 1') and also to cv. 'Hiberna' carrying *rym10* encoding resistance to BaYMV-1 and -2 but susceptibility to the original BaMMV-isolate. On the other hand genotypes with the resistance genes *rym1+rym5*, *Rym2*, *rym4*, *rym7*, *rym8*, *rym9*, *rym11*, *rym12*, $Rym14^{Hb}$, *rym15* and $Rym16^{Hb}$ could not be infected by BaMMV-Teik or BaMMV-Tasl, respectively. Furthermore, 20 accessions of the genebank of the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben being resistant to BaMMV and BaYMV-1/-2, but with up to now unknown genetic background, turned out to be resistant. In 2005 no infection with the *rym5* resistance breaking strain could be detected at Eikeloh and also in 2006 its incidence was very rare. In contrast to the results from Eikeloh, the new strain was detected each year in the small field at Aschersleben. 'Tokyo' was infected at a rate of 5.4% in 2005 and of 23.3% in 2006.

## PP-4_112: PRSV-Helper component proteinase: more than a RNAi suppressor

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HcPro of potyviruses is an established post-transcriptional gene silencing (PTGS) suppressor, besides playing important role in transmission, replication and proteolytic activity. Structural and the functional genomics of the HcPro from different viral origins will help in understanding its differential behavior in RNAi pathway. In view of this, *Papaya ring spot virus* (PRSV) Delhi-HcPro was sequenced and small RNA binding ability of PRSV (Delhi)-HcPro was tested from purified recombinant HcPro protein which suggested its close resemblance with PVY-HcPro in terms of sequence comparison and non-binding of small RNAs. Recently it has been demonstrated that *Tobacco etch virus*-HcPro unlike PVY- HcPro interacts physically with siRNA duplex and acts through siRNA sequestration. Different concepts related to the HcPro function as PTGS suppressor evolved in different studies and in different potyviruses. Various models suggest different mechanisms of its PTGS activity. In one model HcPro was proposed to reverse the established RNA silencing. Another model involved the enlistment of a cellular negative regulator of RNA silencing such as rgs-CaM, a calmodulin related protein. Third model proposed that HcPro acts downstream of an RNA dependent RNA polymerase but inhibits the accumulation of siRNAs suggesting that the Dicer activity was impaired. The fourth model predicted that RISC activation was suppressed through interaction between HcPro and a protein or complex required for the siRNA duplex unwinding. To establish the role of PRSV(Delhi)-HcPro in small RNA metabolism, it was constitutively expressed in *Nicotiana benthamiana,* which resulted morphological aberrations, suggesting its interference with miRNA pathway. These experiments suggested that PRSV(Delhi)-HcPro might interfere with small RNAs pathway by hampering enzymatic machinery and not through the direct binding with small RNAs. Further, co-transformation of PRSV(Delhi)-HcPro with ToLCNDV-AC4 suppressor revealed strong synergistic effects in gene silencing, suggesting two suppressors might be affecting RNAi machinery at two different levels. Besides this, *in silico* domain prediction revealed presence of deubiquitinylation domain, which suggested its involvement in post-translational modifications also. We present here a first report of its probable involvement in virus counter defense, at the level of protein turnover beside the PTGS suppression.

## PP-4_113: Complete nucleotide sequence of *Papaya ringspot virus 'P'* isolate from India

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*Papaya ringspot virus* (PRSV) is a single stranded RNA virus in the genus *Potyvirus* of the family *Potyviridae*. The virus occurs worldwide and is a major constraint to papaya (*Carica papaya L.*) and cucurbit production throughout the tropical and subtropical regions. PRSV genome is well characterized and eleven PRSV isolates, one each from Hawaii and Mexico, two each from Brazil and Thailand and five isolates from Taiwan have been completely sequenced. Though some information on genetic diversity of PRSV population based on coat protein gene has been generated in India., information on the complete nucleotide sequence of PRSV genome is lacking. The complete nucleotide sequence of PRSV-P isolate (PRSV-DEL) was determined from nine overlapping cDNA clones. The viral genome is 10317 nucleotides long, excluding the 3´ terminal poly (A) tail and contains one open reading frame (ORF) starting at nucleotide position 86 and ending at position 10111, followed by a 3´ untranslated region (UTR) of 206 nucleotides. The ORF potentially encodes a polyprotein of 3341 amino acids possessing nine potential clevage sites. Comparative sequence analyses revealed that the PRSV – DEL isolate from India shared an overall 83 – 89% and 90-92% sequence identities at nucleotide and amino acid levels respectively with other PRSV isolates. Maximum sequence identity at amino acid levels (92%) was observed with isolates from Americas forming one cluster, followed by 90-91% identity with Asian isolates, forming distinct cluster. Like other potyviruses, 5′UTR and P1 regions in PRSV genome were highly variable as 64-84% and 67-76% sequence identities at nucleotide and amino acid levels were observed respectively. 3′UTR and other functional regions of the polyprotein of PRSV were highly similar as 89-97% sequence identity both at nucleotide and amino acid levels were observed with other PRSV isolates.

## PP-4_114: Expression of *Cucumber mosaic virus* (CMV) coat protein gene (*Gladiolus isolate*) in tobacco

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Most of the Gladiolus cultivars are infected by both *Cucumber mosaic virus* (CMV) and *Bean yellow mosaic virus* (BYMV). Coat protein gene of CMV was amplified by RT-PCR, cloned in pTZ57R/T vector and sequenced. The sequenced CP gene was ligated in binary vector pBI 121 and introduced in *Agrobacteriurn tumefaciens*. Further, in order to examine the expression of CP gene, the tobacco leaf discs were infected by transformed *A. tumefaciens* and cultured on selection medium (MS + NAA 0.1 + BAP 1.0) containing cefotaxime (500 mg/l) and kanamycin (100mg/l). Kanamycin resistant tobacco plants were regenerated and analyzed by Southern, Northern and Western blot tests. We observed an immune phenotype in seven transgenic lines expressing the CP-CMV protein at different detectable levels. However, in three other transgenic lines, there was delay in symptom appearance upto a frequency of 35 and 70%. Transgene transcripts of the CMV- CP gene were detected by Northern blot in these three lines, but the protein could not be detected by Western blot. The CP was not detected in transgenic lines because the protein may be unstable in plant or the amount of CP produced below the detection level of Western blotting. The possibility that CP gene was not translated in these three lines due to frameshift mutation or some other modification cannot be ruled out and requires further studies. The experimental results will be discussed.

## PP-4_115: Cloning and expression of coat protein gene of *Sweet potato feathery mottle virus* in *E. coli*

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Sweet potato feathery mottle disease caused by *Sweet potato feathery mottle virus* (SPFMV) is widely prevalent in India. Production of disease free healthy planting material demands robust, sensitive and reliable diagnostic methods. Though it is possible to detect virus using RT-PCR, screening of large number of plant samples is difficult. ELISA or DIBA with polyclonal antiserum is the commonly used method for detection of viruses in planting materials on large scale. Polyclonal antiserum production requires purification of the virus SPFMV has limited host range and it is difficult to purify. Hence, attempt was made to clone the coat protein gene of the virus in bacterial expression vector so that the expressed protein could be used for production of polyclonal antiserum. SPFMV coat protein gene was obtained by RT-PCR amplification of total RNA isolated directly from virus-infected sweet potato leaf. The PCR amplified product was cloned in pGEM-T vector and sequenced. The DNA sequencing showed that the cloned SPFMV coat protein gene was 960 bp in size. The sequence obtained was compared with other SPFMV coat protein gene sequences available in the gene bank. Sequences analysis revealed that the SPFMV isolate under study is closely related (98% identical) to SPFMV Egyptian isolate. The coat protein gene was further sub-cloned into bacterial expression vector pET32b and transformed in *E. coli* BL 21 strain. The transformed *E. coli* BL 21 cells were incubated for four hours in LB broth and the protein expression was induced by adding 0.1M IPTG. The expressed protein was further confirmed by DIBA and Western blotting. The result showed that the size of the expressed SPFMV coat protein was 35 kD and the binding of expressed protein with the SPFMV polyclonal antiserum indicated that the bacterial expressed SPFMV coat protein still maintained the native epitope as in the virus. The expressed protein is used for preparing polyclonal antiserum against SPFMV.

## PP-4_116: Detection and characterization of a begomovirus associated with leaf curl disease of ornamental croton (*Codieum variegatum*)

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Leaf curl disease caused by a begomovirus was observed on croton plants, *Codiaeum variegatum* (L.), a popularly grown ornamental plant in botanical, home and office gardens in and around Bengaluru, South India in 2004-05. Virus-infected plants showed typical symptoms of vein thickening, severe inward curling and reduction in leaf size and stunting. The disease was transmitted to healthy croton plants by grafting of infected scions, and through the whitefly vector, *Bemisia tabaci*, suggesting that the disease was caused by a begomovirus. In order to confirm the association of a begomovirus with croton leaf curl disease total DNA was extracted from infected plants and subjected to amplification of viral genome by polymerase chain reaction. DNA fragments of c. 520 bp and 575 bp of coat protein (CP) gene of DNA A were amplified using degenerate primers from diseased plants but not from healthy plants. The 575 bp fragment corresponding to the core region of the CP was cloned and sequenced. Phylogenetic analysis of the core CP sequence of the begomovirus, which we tentatively called, croton leaf curl virus (CrLCuV) with those of selected begomoviruses showed that CrLCuV clustered with *Ageratum yellow vein virus* (AJ810825) and shared the highest nucleotide identities (95%). CrLCuV was equally similar (90-95%) to many other begomoviruses from the Indian sub-continent that infects tomato, tobacco, cotton and papaya, thus its precise taxonomic denomination requires sequencing complete viral genome.

## PP-4_117: PRSV infection on chimeric transgenic (T0) papaya scores tissue for gene integration

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Plant chimeras have been a valued resource for biologists to trace the developmental fate of individual cells in a meristem. The generation of genetic mosaics often involves fusion of meristems of two species with distinct phenotypic differences at the cellular level. *In-vivo* electroporation of an apical meristem with DNA results in a certain proportion of cells being stably transformed. The resulting vegetative shoots as well as floral organs from such meristems are genetic chimeras. Scoring of individual chimeric tissues is dependent on the phenotypic differences that the introduced gene may confer. In the present study, papaya cultivar Solo was transformed by *in-vivo* electroporation of apical meristems of 2 month old seedlings with a plant expressible gene construct which confers resistance to *Papaya ring spot virus* (PRSV). The plants were grown to maturity for a period of about 2 years before challenging thrice with PRSV using viruliferous aphids. Scoring for symptoms was carried out 3 months after the first challenge. Chimeric leaves and branches were obtained with large sections that were completely free from the virus. While the field-infected plants under natural conditions do exhibit apparently healthy regions within a leaf, especially under moderate levels of infection, the present level of challenge resulted in severe infection in non-transgenic controls. The chimeric transgenic plants had a whole branch or more than half a lamina completely free from the virus. We present molecular evidence for the presence of the transgene and for the absence of the virus in such regions. This is the first report of use of both a transgenic chimera and a virus to phenotypically score transgenic and non-transgenic sectors towards understanding the developmental origin of leaves and whole branches of papaya.

## PP-4_118: Aphid vectors and transmission of Potato virus Y strains

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In the last 5 years Dutch seed potato growers face increasing problems with *Potato virus Y* (PVY). Seed potato lots which have too high virus percentages in the post harvest control are declassified into lower quality classes. This declassification leads to high economic losses. The increasing problems with PVY seem to be in contradiction to the aphid populations in the Netherlands. Over the past 20 years the number of aphids caught by yellow water traps or high suction traps decreased dramatically. This also goes for *Myzus persicae,* which is considered to be the most efficient vector of PVY. In the Netherlands the control system for PVY is based on monitoring virus infections in the field, the flight data of a selected group of aphids and post-harvest control by ELISA. The aphids caught are counted and assigned a value according to their Relative Efficiency Factor (REF). When the cumulative values reach a certain threshold a date for haulm destruction is set, to prevent virus infections of the tubers. Given the current problems with PVY, this system, developed in the 1980's, may no longer be sufficient to control the current virus situation in the field. In our research the question is addressed whether the REF's determined in the 1980's are still valid for *M. persicae* and other aphids for the PVY strains found in the Netherlands nowadays. A new system was set up to determine the REF's for known PVY transmitters and other aphids that are presently caught in the field. Figures of transmission efficiencies of different aphid species for different PVY strains will be presented.