

**3<sup>rd</sup> International Symposium**  
***Phytophthora: Taxonomy, Genomics,  
Pathogenicity, Resistance and  
Disease Management***

**9-12 September 2015  
Bengaluru, India**

**ABSTRACTS**

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**3<sup>rd</sup> International Symposium**

**on**

***Phytophthora : Taxonomy, Genomics,  
Pathogenicity, Resistance and  
Disease Management***

**9-12 September 2015**

**ABSTRACT**

Sponsored by

Department of Science and Technology  
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Indian Council of Agricultural Research  
Department of Horticulture, Govt. of Karnataka  
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ICAR-Central Plantation Crops Research Institute  
ICAR-Indian Institute of Horticultural Research  
Association for Advancement of Pest Management in Horticultural Ecosystems



Abstracts: 3<sup>rd</sup> International Symposium on *Phytophthora* :  
Taxonomy, Genomics, Pathogenicity, Resistance and Disease Management,  
ICAR-IIHR, Bengaluru, 9-12, September, 2015 p. 75.

Published by

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August 2015

Cover design & Layout

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Printed at

Print Express, Kaloor, Kochi



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भारतीय कृषि अनुसंधान परिषद  
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## MESSAGE

I am happy to learn that ICAR-Central Plantation Crops Research Institute, Kasaragod, ICAR-Indian Institute of Horticultural Research, Bangalore and Association for Advancement of Pest Management in Horticultural Ecosystems, Bengaluru are jointly organizing 3<sup>rd</sup> International Symposium on 'Phytophthora: Taxonomy, Genomics, Pathogenicity, Resistance and Disease Management' during 9-12 September, 2015 at Indian Institute of Horticultural Research, Bengaluru.

*Phytophthora*, "Plant destroyer", is known worldwide for the devastating plant disease epidemics caused and continuing even after 170 years of great potato late blight epidemic in Ireland. It represent a significant and emerging bio-security threat, in large part due to increase in plant movement via international trade.

It is hoped that the discussions during International symposium will help in evolving new research strategies for combating invasive and emerging clonal lineages of this destructive plant pathogen for enhancing food production in an era of declining natural resources and climate change.

I wish the Symposium, a great success.

( S. Ayyappan )

Dated the 20<sup>th</sup> July, 2015  
New Delhi





डा. एन. के. कृष्ण कुमार

उप महानिदेशक (बाग. वि.)

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DEPUTY DIRECTOR GENERAL (HORTICULTURAL SCIENCE)

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KRISHI ANUSANDHAN BHAVAN - II,

PUSA, NEW DELHI 110 001

## MESSAGE

Horticulture in India has made rapid strides since last two decades and touched 268 million tons production in 2013-14. However, there is growing concern to enhance productivity without compromising sustainability amidst declining factors of production and enhanced biotic & abiotic stresses in future. One such limitation are *Phytophthora* diseases. The crop loss caused by *Phytophthora* on various horticultural crops has increased over the years as influenced by evolution of virulent races and fungicide resistance. The problem is likely to get accelerated with introduction of susceptible genotypes and global trade. It calls for renewal of our efforts to understand the problems associated with *Phytophthora* in holistic manner and to chalk a future course of action.

I am happy to learn that the Central Plantation Crops Research Institute (CPCRI), Kasaragod; the Indian Institute of Horticultural Research (IIHR), Bengaluru and the Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE), Bengaluru are jointly organizing the third International Symposium on *Phytophthora*: Taxonomy, Genomics, Pathogenicity, Resistance and Disease Management” during 9-12 September, 2015 at IIHR, Bengaluru. I hope that the event will provide a common platform to researchers across the globe to deliberate upon the dynamics of *Phytophthora* diseases and suggest way forward for its management.

I compliment the organizers and wish the participants a fruitful symposium.

**(N. K. Krishna Kumar)**







भाकृअनुप-केन्द्रीय रोपण फसल अनुसंधान संस्थान  
कासरगोड़ - 671124, केरल, भारत  
ICAR-Central Plantation Crops Research Institute  
Kasaragod 671124, Kerala, India



## About the Symposium and Welcome Message

I have immense pleasure in extending warm welcome and wishing a comfortable stay at Bengaluru, garden city or knowledge or silicon city of India to the distinguished delegates of the International Symposium on *Phytophthora*: Taxonomy, Genomics, Pathogenicity, Resistance and Disease management during 9-12, September, 2015 at Indian Institute of Horticultural Research, Bengaluru.



Stramenopile pathogens of the genus *Phytophthora* cause devastating diseases on a wide range of agricultural crops, natural vegetation and forestry worldwide. There are over 120 species in the genus and many have a wide host range. *Phytophthora infestans* that caused the Great Irish potato famine still remains the most destructive pathogen of potatoes and tomatoes, causing crop losses up to \$ 6.7 billion annually. Other notable species that have emerged in more recent times are *P. ramorum*, a huge exotic pathogen causing sudden oak death, *P. alni* responsible for devastating losses to alder stands across Europe, *P. kernoviae*, a major pathogen of ornamental species in Europe, *P. cinnamomi* responsible for a major epidemic of forest in Australia, *P. agathis* affecting kauri in New Zealand, *P. cactorum* causing bleeding canker in hardwood trees, *P. capsici* infecting peppers and cucurbits, *P. fragariae* affecting strawberries, *P. megakarya* causing huge cocoa crop loss in Africa, *P. palmivora* killing palms in south east Asia and India and *P. meadii* devastating arecanut, small cardamom, nutmeg and rubber in India.

Despite myriad of important discoveries made, *Phytophthora* continues to be a major pathogen and needs future research directions to develop cost effective and durable management strategies. Plant trade and poor implementation of quarantine regulations have led to serious disease outbreaks including potato late blight which spread by movement of infected tubers and sudden oak death which has moved through infected nursery plants. The movement of infected planting materials pose severe bio-security problems which need to be addressed to avoid chance of introduction of *Phytophthora* at global level.

This symposium provides a platform to deliberate the issues of global importance, prepare action plan and develop global initiative on *Phytophthora* research to contain the threats posed by this pathogen. I profusely thank Dr. S.Ayyappan, Secretary, DARE, Government of India and Director General, Indian Council of Agricultural Research, New Delhi for his constant support and guidance. My thanks are also due to Dr. N.K.Krishna Kumar, Deputy Director General (Hort.Sci.) Indian Council of Agricultural Research, New Delhi for his encouragement.

**P. Chowdappa**  
Director

ICAR-Central Plantation Crops Research Institute  
Kasaragod-671 124, Kerala



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3<sup>rd</sup> International Symposium on  
***Phytophthora* : Taxonomy, Genomics, Pathogenicity, Resistance and  
 Disease Management**

**9 - 12, September, 2015**

**Inaugural Function**

**09-09-2015/Wednesday/10.00-11.30hrs**

9.00-10.00	: Registration
10.00	: ICAR song
10.02	: Invocation and lighting lamp
10.05	Welcome Address : Director Indian Institute of Horticultural Research, Bengaluru, India
10.10	About Symposium : Dr. P. Chowdappa Director & Organizing Secretary Central Plantation Crops Research Institute, Kasaragod, India
10.15	Address by Guests of Honour : Dr. William E. Fry Plant Pathology and Plant-Microbe Biology Cornell University, Ithaca NY 14853 USA  Dr. N.K. Krishna Kumar Deputy Director General (Hort. Science) ICAR, New Delhi, India
10.50	Release of publications : Indian Horticulture Indian Spices <i>Phytophthora</i> - An Indian Perspective Book of Abstracts
11.00	Inaugural address by Chief guest : Dr. S. Ayyappan Secretary, DARE, Govt. of India Director General, ICAR, New Delhi, India
11.20	Vote of thanks : Dr. M. Krishna Reddy Head, Division of Plant Pathology, IIHR, Bengaluru and The President AAPMHE, Bengaluru, India
11.30	: High Tea
12.00-13.00	: Keynote lecture : <i>Phytophthora</i> : An Indian Perspective Dr. P. Chowdappa, Director ICAR-CPCRI, Kasaragod, India
13.00-14.00	: Lunch

**Session 1****Emerging diseases and impact in horticulture, forestry and natural ecosystems****09-09-2015/Wednesday/14.00-16.00hrs**

Chairperson : **Andre Drenth**  
Co-Chairperson : **M. Anandaraj**  
Convenor : **Vinayaka Hegde**

**Plenary lecture**

Current and emerging *Phytophthora* diseases – research challenges and impact-André Drenth

**Lead Presentation**

Recent advances in understanding and management of late blight disease of potato  
B.P. Singh, Sundaresha S. and Sanjeev Sharma

Severe outbreaks of foliar blights and fruit rots on vegetable crops in India caused by invasive and emerging *Phytophthora* populations- P. Chowdappa

Characterization of *Phytophthora* spp. causing outbreaks of leaf and nut fall of Nutmeg (*Myristica fragrans* Houtt) in Kerala- M. Anandaraj , Sally K. Mathew, R. Suseela Bhai, Santhosh J. Eapen, J. Cissin and B. Rosana.

**Oral Presentation**

First finding of *P. nicotianae* on *Philodendron xanadu* and *P. citrophthora* on *Nerium indicum* as well as first report of *P. nicotianae* on *Spathiphyllum* spp. in India- R. Dhali and S. Guha Roy

Tea break: 16.00-16.20 hrs

**Session 2****Taxonomy and phylogeny****09-09-2015/Wednesday/16.20-18.00hrs**

Chairperson : **D.E.L. Cooke**  
Co-Chairperson : **P. Chowdappa**  
Convenor : **A. K. Das**

**Plenary lecture**

Evolutionary origins of US and famine-era lineages of *Phytophthora infestans*- Amanda Saville, Michael D. Martin, Tom Gilbert and Jean Ristaino

**Lead Presentation**

Taxonomic investigations warrants re-description of *Phytophthora* species infesting black pepper in India- M. Anandaraj, R. Suseela Bhai, Santhosh J. Eapen, R. Praveena., Bharath Nair, Cissin Jose and O.B. Rosana .

**Oral Presentation**

PhytoWeb: A Database for *Phytophthora* Diseases of Horticultural Crops- Santhosh J. Eapen, Alias Eldhose, S, Balaji., T.K. Vinod and M. Anandaraj

**Session 3****Population biology and gene flow  
10-09-2015/Thursday/10.00-12.00hrs**

Chairperson	:	<b>Jean Ristaino</b>
Co-Chairperson	:	<b>Y.R. Sarma</b>
Convenor	:	<b>Sanjeev Sharma</b>

**Plenary lecture**

New tools to understand evolving populations of *Phytophthora infestans* on regional, national and international scales for improved late blight management- Cooke D.E.L., Kessel G.J.T., Lees A.K., Lassen P. Baby S., and Hansen J.G.

**Lead Presentation**

Towards understanding the extent of genetic diversity of *Phytophthora* spp. infecting rubber (*Hevea brasiliensis*) and deploying molecular markers for resistance breeding-C. Bindu Roy and T. Saha

Tea break 11.00-11.20hrs

**Oral Presentation**

Population structure of *Phytophthora infestans* infecting potato and tomato in India -Nirmal B., J. Kumar and P. Chowdappa

Genetic structure, aggressiveness and fungicidal sensitivity of *Phytophthora* associated with foliar blights of hot pepper- S. Madhura, P. Chowdappa and K.V. Pavani

Seasonal population fluctuation of *Phytophthora nicotianae* var. *parasitica* in Kinnow mandarin orchards under Punjab conditions-Sarbjeet Kaur, Umesh Kumar Dhakad and S.K. Thind

Genetic diversity of *Phytophthora infestans* isolates on tomato and potato from Eastern India- T.Dey, S.Tewari, K.L. Myers, S. Tripathi, W.E. Fry and S. Guha Roy

Lunch Break: 12.00-13.00 hrs

**Session 4****Genetics and genomics****10-09-2015/Thursday/13.00-15.00hrs**

Chair Person	:	<b>Brett M. Tyler</b>
Co-Chairperson	:	<b>Francine Govers</b>
Convenor	:	<b>M.L. Jeeva</b>

**Plenary lecture**

Genome evolution in filamentous plant pathogens- Sophien Kamoun

**Lead Presentation**

Comparative genomics for identification of orthology and phyletic patterns in *Phytophthora* sp.- Monica Chandran, Rosana O.B., Santhosh J. Eapen and M. Anandaraj

**Oral Presentation**

A species- specific PCR for detection of *Phytophthora meadii*, the causal agent of fruit rot of arecanut - Sharadraj K.M., Nidhina K., Gangaraj K.P., Smita Nair, Prathibha V.H.\* and Vinayaka Hegde.

Comparative genome analysis of Irish Famine pathogen with Indian *Phytophthora infestans* isolate Sundaresha, S., Sanjeev Sharma, B.P. Singh, Jeevalatha A., Shashi Rawat, Ajay Kumar Mahota and T.R. Sharma

An efficient method for zoospore production, infection and real-time quantification of *Phytophthora cajani* causing *Phytophthora* blight disease in pigeonpea- M. Sharma, R. Ghosh, and A. Tarafdar

Improved Genome assembly of *Phytophthora ramorum* by third generation sequencing technology- Mathu Malar C., Jennifer Yuzon, Takao Kasuga and Sucheta Tripathy

Tea break: 15.00-15.20hrs

**Session 5****Pathogenesis and plant innate immunity****10-09-2015/Thursday/15.20-17.00hrs**

Chair Person	:	<b>Sophien Kamoun</b>
Co-Chair person	:	<b>M. Babadoost</b>
Convenor	:	<b>Bindu Roy</b>

**Plenary lecture**

From genomics to effectors: how understanding oomycete biology has contributed to disease control- Brett Tyler

**Lead Presentation**

Transcriptome assisted label-free proteomic analysis of *Phytophthora capsici* x *Piper nigrum* L.





phytopathosystem-M. Chidambareswaren, K. Anu and S. Manjula

Incompatible Interactions of *Piper colubrinum* with *Phytophthora capsici*: Insights from gene expression studies - K. Johnson George, I.P. Vijesh Kumar, Neema Malik, Rosana Babu, Santhosh Eapen J and M. Anandaraj

### Oral Presentation

*Medicago truncatula* genes implied in Mycorrhiza and *Phytophthora palmivora* infection identified through genome-wide association studies - A. Chatterjee, T. Rey, M. Bonhomme, J. Toulotte, C. Jacquet and S. Schornack

Towards understanding the black pepper-*Phytophthora* pathosystem - using integrated transcriptome and proteome datasets- P. Umadevi, Johnson K. George and M. Anandaraj

Nodal inoculation a quick and easy inoculation technique for *Phytophthora* blight of pigeonpea- Ramesh Chand, Vineeta Singh, Chandra Kant Singh, Raju Ghosh and Mamta Sharma

## Session 6

### Epidemiology and decision support systems

11-09-2015/Friday/10.00-11.00hrs

Chair person	:	William E. Fry
Co-Chairperson	:	S. Guha Roy
Convenor	:	Biju C.N.

### Plenary lecture

Epidemiology and Decision Support Systems - William E. Fry, Ian M. Small and Giovanna Danies.

### Lead Presentation

Indoblighcast- A simple generic DSS for Late blight management - P.M. Govindakrishnan, B.P. Singh, Islam Ahmad, Shashi Rawat and Sanjeev Sharma

Forecasting of late blight in India- A success story - R.K. Arora

### Oral Presentation

Global potential distribution of *Phytophthora infestans* under current and climate change situations - V. Sridhar, L.S. Vinesh, S. P. Mohan Kumar, P. Chowdappa and A. K. Saxena.

Survey and surveillance of *Phytophthora* rot of cocoa in Southern transition zone of Karnataka Jayalakshmi, K., Nagarajappa Adivappar, Narasimhamurthy, H.B., Ravindra, H., Raju, J., and Soumya D. M.

Tea break: 11.00- 11.20

## Session 7

### Host plant resistance, molecular breeding and variety development 11-09-2015/Friday/11.20-13.30hrs

Chair person	:	<b>D.E.L. Cooke</b>
Co-Chairperson	:	<b>Elizabeth Dann</b>
Convenor	:	<b>I.M. Sharma</b>

#### Plenary lecture

Potato late blight in developing countries, intensity, management and future risks - Gregory A. Forbes

#### Lead Presentation

*Piper colubrinum*, resistant root stock as an option for eco-friendly disease management of *Phytophthora* foot rot of black pepper (*piper nigrum* L) caused by *Phytophthora capsici* Leonian - Y.R. Sarma , K. Nirmal Babu , L.N. Hegde, Homey Cheriyan and P. Jayaraj

Crop resistance improvement by mining natural and induced variation in host accessibility to *Phytophthora palmivora* -S. Schornack

#### Oral Presentation

Effect of plant age and cultivar on Population of *Phytophthora nicotianae* var. *parasitica* causing foot rot/gummosis in Kinnow mandarin and Sweet orange- Umesh Kumar Dhakad, Sarbjeet Kaur and S.K. Thind

Identification and *in silico* characterisation of putative resistance genes in black pepper and related species - E.J. Suraby, O.B. Rosana, K.G. Johnson, D. Prasath, S.J. Eapen, K.N. Babu And M. Anandaraj

Identification of sources of resistance against *Phytophthora* in arecanut - Prathibha, V.H., Vinayaka Hegde, Sharadraj, K.M, Nidhina, K, Nagaraja, N.R and Chaithra M.

Managing late blight disease of potato through introgression of RB gene in Indian potato cultivars - Sanjeev Sharma, B.P. Singh, Sundaresha S., Vinay Bhardwaj and S.K. Kaushik

Lunch Break 13.30- 14.30



## Session 8

### Disease management- Fungicide and Biological control 11-09-2015/Friday/14.30-18.00hrs

Chairperson : David I. Guest  
Co-Chair person : Elizabeth Dann  
Convenor : R. Suseela Bhai

#### Plenary lecture

Management of *Phytophthora* diseases in the tropics- David I. Guest

#### Lead presentation

Management of *Phytophthora* blight (*Phytophthora capsici*) of peppers- M. Babadoost, D. Tian, S.Z. Islam, Ande. Pavon

The 30 year journey: Integrated *Phytophthora* root rot management in avocados- Elizabeth Dann, Ken Pegg, David Armour, Tony Whiley

In search for new targets to control *Phytophthora* pathogens- Francine Govers

Tea break : 16.00-16.20

#### Oral presentation

*Trichoderma* based Eco-friendly management of *Phytophthora* diseases - Pratibha Sharma, Manika Sharma and M. Raj

Strategic management of root knot nematode induced *Phytophthora* disease complex in horticultural crops through biopesticides- M. S. Rao, R. Umamaheswari, P. Prabu, K. Priti, M. Kamalnath and R. Manoj Kumar

Development of bio-control formulations for management of late blight of tomato- S.P.Mohan Kumar and P. Chowdappa

New vistas in management of *Phytophthora* in black pepper (*Piper nigrum* L.)-Sreeja K., Suseela Bhai R. and Anandaraj M.

Organic management of taro leaf blight incidence caused by *Phytophthora colocasiae*- S.S. Veena, M.L. Jeeva, J. Sreekumar and S.K. Chakrabarti

Bio-efficacy of endophytic *Trichoderma* species against *Phytophthora palmivora* the causal agent of black pod disease of cocoa - M. Chaithra, Prathibha, V.H., Apshara, E.S., Vinayaka Hegde

An efficient and eco-friendly tool for sustainable management of taro leaf blight (*Phytophthora colocasiae*) - B.K.M. Lakshmi, Padmaja, Umadevi, Vijaya M. and Hari Prasad N.

Studies on efficacy of fungicides and their dosage against *Phytophthora colocasiae* causing leaf blight of Taro- G. Padmaja, B. Kanaka Mahalakshmi, G. Uma Devi and D. Sridevi

Fungicide sensitivity of *Phytophthora infestans* isolates on tomato and potato from Eastern

India.- T. Dey, S. Tewari, W.E. Fry, S. Tripathi and S. Guha Roy

Integrated management of black shank disease of FCV tobacco (*Nicotina tabaccum* L.) in field and its effect on leaf yield and quality parameters - C. Karegowda, H. Narayan Swamy, J. Raju, H. G. Sharanappa, R. Ganesh Naik and R. Murali

Management of fruit rot of arecanut caused by *Phytophthora meadii* under *in vivo* conditions - H. Narayan Swamy, J. Raju, H. G. Sharanappa, R. Murali and Shrinidhibharathisha Navilekar

Biological management of potato late blight disease using *Bacillus subtilis* var. *amyloliquefacians*- U. Keerthana, K. Nagendran, T. Raguchander, L. Rajendran and G. Karthikeyan

Evaluation of bioagents for managing *Meloidogyne incognita* and *Phytophthora* disease complex in capsicum (*Capsicum annuum* L. var. *grossum* Sendt) under protected conditions- R. Umamaheswari, M. S. Rao, R. Rajinikanth, M. K. Chaya, G. N. Grace and Vidyashree

Management of late blight disease in kharif potato at Karnataka - E.P.Venkatasalam, A. Bairwa, R. Sudha, R. Umamaheswari and B.P. Singh

Management of *Phytophthora parasitica* causing Heart rot of Pineapple-K.R. Shreenivasa., D. Rekha and B.C. Hanumantha Swamy

Field efficacy of new fungicide molecules against late blight of potato in Southern - Karnataka. Anjaneya Reddy, B., Sreenath, A., Rekha, D. and Devappa, V.

*Phytophthora* root rot of *Moringa oleifera* and its management- Girija Ganeshan, Archana S. Rao and Lakshmi K.P.

Status and Management of *Phytophthora* diseases in Coorg Mandarin - P.S. Sonavane, A.K. Saxena, V.Venkataravanappa, M. Krishna Reddy and Jayanthi Mala

Evaluation of chemicals and bioagents for managing *Phytophthora* root rot and root-knot nematode disease complex in capsicum under protected cultivation - J. Raju, Nagarjappa Adivappar, K. Jayalakshmi, H. Ravindra, H. B. Narasimhamurthy And D. M. Soumya.

Evaluation of chemicals for managing *Phytophthora capsici* and *Meloidogyne incognita* disease complex in Black pepper under poly house conditions - Jayalakshmi, K., Ravindra, H., Soumya, D. M., Narasimhamurthy, H. B., Nagarjappa Adivappar and Raju J.

## Session 9

### Workshop on oomycete databases 12-09-2015/Saturday/10.00-12.00hrs

Chairperson	:	Brett M. Tyler
Co-chairperson	:	P. Chowdappa
Convenor	:	Sucheta Tripathy

FungiDB: An integrated functional genomics database for fungi and oomycetes – Brett M. Tyler, Edward Liaw, Dai Gu, Venkatesh Moktali, Sufen Hu, Haming Wang, John Brestelli, Debbie





Pinney, Omar Harb, Brian Brunk, Jessica C Kissinger, David Roos, Jason E. Stajich

Tea : 11.00-11.20

EuMicrobedb: A genome database and annotation interface system for Oomycetes pathogens.-  
Arijit Panda, Akash Gupta, Gyan Prakash Mishra, Madhavi Madduluri, Mathu Malar C ,  
Deeksha Singh, Brett M. Tyler\* and Sucheta Tripathy

## Session 10

### Interactive meeting between scientists and stakeholders

**12-09-2015/Saturday/12.00-14.00hrs**

Chairperson	:	<b>Y.R. Sarma</b>
Co-chairperson	:	<b>P. Chowdappa</b>
Convenor	:	<b>Santhosh J. Eapen</b>

DuPont™ Zorvec™ (“DPX-QGU42”, “oxathiapiprolin”): The first member of a novel class of oomycete fungicides- C.P. Shepherd, W.J. Summers, J.A. Bruhn T.H. Carski, J.A. Sweigard and Ashutosh Bhaik

Integrated management of *Phytophthora* diseases of cocoa- R.ChandraMohan

Managing fungicide resistance in *Phytophthora* caused plant diseases-T.S. Thind

*Phytophthora* diseases of apple and their management in India-I.M. Sharma

*Phytophthora* diseases of citrus in India-A. K. Das

*Phytophthora* diseases of cassava and taro- M.L. Jeeva, S.S. Veena, Vishnu S.Nath, Senthil @ Sankar, B. Shyni, and R.S. Misra

*Phytophthora* diseases of spice crops-M. Anandaraj

*Phytophthora* diseases of vegetable crops-P. Chowdappa

*Phytophthora* diseases of palms- Vinayaka Hegde and P. Chowdappa

*Phytophthora* diseases in ornamental crops in India- S. Sriram

*Phytophthora* diseases of rubber-Bindu Roy

Zorvec™ an effective fungicide for the management of downy mildew in grapes in South Asia -  
Ashutosh Bhaik, Pampapathy Gurulingappa and Rajiv Rathod

Zorvec™ a new age fungicide for the management of late blight in Potato in South Asia -  
Ashutosh Bhaik and Rajan Trivedi

Lunch break: 14.00-15.00

**Plenary session**  
**12-09-2015/Saturday/15.00-17.00hrs**

Chairperson	:	<b>Sophien Kamoun</b>
Co-Chairperson	:	<b>P. Chowdappa</b>
Convenor	:	<b>S. Sriram</b>

15.00- ICAR song

15.05- Invocation and lighting lamp

15.10- 16.10

Presentation of session reports and recommendations

Adoption, resolution and declaration on *Phytophthora*

16.10- Remarks by Chairperson

17.00- National Anthem



## Session: 1

# Emerging diseases and impact in horticulture, forestry and natural ecosystems

## Current and emerging *Phytophthora* diseases – research challenges and impact

André Drenth

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India is the second largest producer of vegetables in world with an annual production of 87.53 million tonnes from 5.86 million hectares, accounting 14.4% to the world production. Severe outbreaks of *Phytophthora* diseases such as late blight on tomato and potato, fruit rot on brinjal tomato and cucurbits, foliar blights in hot pepper were recorded in India since 2008. *Phytophthora infestans* isolates collected from potato and tomato were A2 mating type, metalaxyl resistant, mtDNA haplotype Ia and had RG57 and SSR fingerprints almost identical to the 13\_A2 clonal lineage reported in Europe. All isolates on detached leaflets of three potato and tomato cultivars showed to be equally aggressive, confirming that the same clonal population is infecting both hosts. *Phytophthora* isolates from hot pepper were identified majority of the isolates as *P.boehmeriae* and few isolates as *P.capsici* based on morphological and molecular criteria. All isolates of *P. boehmeriae* were metalaxyl sensitive while *P.capsici* isolates were intermediate in sensitivity. *P.boehmeriae* isolates were highly aggressive than those of *P. capsici* isolates.

*Phytophthora nicotianae* isolates from brinjal and ridge gourd isolates were A2 while tomato isolates were both A1 and A2. All isolates were metalaxyl sensitive. Isolates were genotyped for three mitochondrial (*rpl5-rns*, *rns-cox2* and *cox2+spacer*) and three nuclear loci (*hyp*, *scp* and *β-tub*). All genes were polymorphic but nuclear genes were more variable than mitochondrial genes. The network analysis of genotypes using the combined data set of three nuclear genes revealed a host specific association. *P. nicotianae* isolates were highly aggressive and produced significantly larger lesions on their respective host than on their alternative host. Revised management strategies will be required to combat these destructive pathogens and monitoring of the population across other vegetable -growing regions of India is warranted.

## Recent advances in understanding and management of late blight disease of potato

B.P. Singh\*, S. Sundaresha and Sanjeev Sharma

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The *Phytophthora infestans* is one of the most confounding pathogen and model organism that exhibits high evolutionary potential and rapidly adapts to changing environment. This fungus

like organism is responsible for global annual crop loss of US\$ 12 billion. Despite potato resistance breeding, fungicide use, and other control measures, it still continues to be a major threat to sustainable potato production worldwide. *P. infestans* population has undergone drastic change during the last two decade. New population has emerged which is more diverse having more pathotypes, carrying the new mating type (A<sub>2</sub>), haplotype etc. The whole genome of *P. infestans* (240Mb) has been sequenced which is several fold larger than those of related species like *P. sojae* (95 Mb) and *P. ramorum* (65 Mb). It has been proved beyond doubt that the dynamism in *P. infestans* is mainly due to the movement of transposable elements which covers 74% of the genome. During interactions, both host and pathogen secrete complex proteins for surveillance, assault, and defence and counter defence activities. Similarly, the potato genome (845Mb) containing 39045 protein coding genes and more than 700 R-genes have been identified which play crucial role in understanding host-pathogen interaction. Advances in science of molecular biology, high-throughput analytical disciplines like genomics, transcriptomics, proteomics etc. have emerged to gain deep knowledge about the secretome in understanding and managing the pathogen and the disease. Several hypotheses demonstrated that *P. infestans* RXLR effectors are candidate *Avr* genes and are functionally profiled on *Solanum* to detect corresponding R genes. Recently, European groups have identified the RXLR (520) and CRINKLER (419) effectors and 19 novel pathogenicity genes including unexpected hypothetical genes from the whole 240 Mb genome architecture through microarray. This has opened new vistas for developing resistant varieties through molecular approach. In India, CPRI has identified and cloned 10 defense genes from the LB resistant Kufri Girdhari cultivar and five pathogenicity genes from *P. infestans* using LB susceptible cultivar through cDNA microarray analysis. RNA sequencing studies have shown expression of defence genes in the background of RB transgenics. Similarly, CPRI demonstrated RB regulated defense system in Kufri Jyoti RB transgenic hybrids and identified R and R- like genes in leaf tissues through cDNA microarray. Based on transcriptomic analysis, we have demonstrated the pathogenicity of effector genes through dsRNA transfection studies which would be used for development of RNAi based fungicide against *P. infestans*. Based on comprehensive analysis, RNAi transgenics have been developed by using *P. infestans Avr 3a* gene. It is now evident that knowledge of omics is essential to understand the secretome of both host and pathogen to develop suitable management strategies.

## **Severe outbreaks of foliar blights and fruit rots on vegetable crops in India caused by invasive and emerging *Phytophthora* populations**

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India is the second largest producer of vegetables in world with an annual production of 87.53 million tonnes from 5.86 million hectares, accounting 14.4% to the world production. Severe outbreaks of *Phytophthora* diseases such as late blight on tomato and potato, fruit rot on brinjal tomato and cucurbits, foliar blights in hot pepper were recorded in India since 2008. *Phytophthora*





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### **Characterization of *Phytophthora* spp. causing outbreaks of leaf and nut fall of Nutmeg (*Myristica fragrans* Houtt) in Kerala**

Anandaraj M<sup>1</sup>, Sally K Mathew<sup>2</sup>, Suseela Bhai R<sup>1</sup>, Santhosh J Eapen<sup>1</sup>, Cissin J<sup>1</sup> and Rosana B<sup>1</sup>

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Nutmeg (*Myristica fragrans* Houtt.) is one of the major spice crops cultivated as an intercrop in coconut and arecanut gardens in many parts of Kerala, India. It is known for the two important spices namely nutmeg and mace. Nutmeg is the dried Kernel of the seed and mace is the dried aril surrounding the seed. The plant is susceptible to a number of fungal diseases. During the monsoon period of 2011, occurrence of a serious leaf and nut fall was observed in major nutmeg growing areas of Kerala viz. Thrissur, Ernakulam, Idukki, Kottayam and Kozhikode. The disease is characterized by severe defoliation and nut fall. Symptoms appeared as dark brown water-soaked lesions on the petiole of the leaves which extends and spread along the lamina resulting in blighting. In order to get correct identity of the causal organism, a detailed study on morphology, temperature requirement and molecular characterization using ITS,  $\beta$ -tubulin, 28SrDNA, Elongation factor 1 $\alpha$  and heat shock protein90 was done. The morphological features of *Phytophthora* isolates of nutmeg (13-01 to 13-06, 13-55 and 98-68) include papillate/semi papillate, ovoid to obovoid sporangia with pedicel length ranging from 14.39- 68.07. The oogonia are amphigynous and formed on pairing with *P. meadii* from cocoa which is of A2 mating and hence the nutmeg isolate is found to be of A1 mating type. All the isolates grow between 15-

30°C with optimum temp 25°C. ITS analysis shows that out of the eight isolates six isolates (13-01,13-02,13-03,13-04,13-05 and 13-06) showed close similarity to *Phytophthora meadii* and two isolates (13-55 and 98-68) to *P. tropicalis*. However, MLST analysis using five genes, it is found that *Phytophthora* isolates from nutmeg form three separate groups in clade 2 indicating species diversification. In SSCP analysis the isolates showed a separate group distinct from *P. meadii* infecting cocoa and cardamom.

**First finding of *P. nicotianae* on *Philodendron xanadu* and *P. citrophthora* on *Nerium indicum* as well as first report of *P. nicotianae* on *Spathiphyllum* spp. in India**

R. Dhali and S. Guha Roy\*

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Here we report presence of *Phytophthora nicotianae* and *Phytophthora citrophthora* on two exotic but common ornamental hosts, *Philodendron xanadu* (Xanadu), and *Nerium indicum* respectively from Eastern India which increases the hitherto known host range of these *Phytophthora* spp. worldwide. We also present a first report of *P. nicotianae* on *Spathiphyllum* spp. in India. The identities of the isolates were determined by morphological methods, ITS-RFLP and partial sequencing of the rDNA ITS region and the sequences submitted to GenBank. Koch postulates performed confirmed the casual organisms which caused leaf blight in *Spathiphyllum* spp. and leaf rot in *Philodendron xanadu* as *Phytophthora nicotianae* and shoot die back of leaf and stem in *Nerium indicum* as *Phytophthora citrophthora*. This is a first finding of *Phytophthora nicotianae* on *Philodendron xanadu*, and *Phytophthora citrophthora* on *Nerium indicum* worldwide. It is also a first report of *Phytophthora nicotianae* on *Spathiphyllum* sp. in India.



## Session: 2

### Taxonomy and phylogeny

#### Evolutionary origins of US and famine-era lineages of *Phytophthora infestans*

Amanda Saville<sup>1</sup>, Michael D. Martin<sup>2</sup>, Tom Gilbert<sup>3</sup> and Jean Ristaino<sup>1\*</sup>

<sup>1</sup>North Carolina State University, Raleigh, NC 27695 2 Center for Theoretical and Evolutionary Genomics,

<sup>2</sup>Department of Integrative Biology, University of California, Berkeley, CA, and <sup>3</sup>Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5–7, 1350 Copenhagen K, Denmark.

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*Phytophthora infestans*, causal agent of the Irish potato famine, is a threat to food security globally and an important pathogen in the southeastern US on both tomato and potato. USA Blight ([www.usablight.org](http://www.usablight.org)) was launched in 2011, and lineages in the US have been genotyped. The mefenoxam sensitive US-22, more common on tomato in 2009, has been displaced by US-23, which infects both hosts. The genetic relationships of 18 of the 24 clonal lineages found in the US since the 1970s were examined using 12 simple sequence repeat (SSR) markers. The US-1 lineage formed a cluster distinct from most modern US lineages in DPCA and STRUCTURE analyses of SSR loci. Five clusters of modern US lineages were identified, and some recent US lineages showed similarity to Mexican lineages with the exception of US-1 and US-23. Mitochondrial haplotype data, SSR and NGS data from herbarium specimens were used to track the emergence of lineages. The US-1 (Ib) lineage increased in frequency in the mid-20<sup>th</sup> century in the US and globally and then declined. Populations from European and US 19<sup>th</sup> century outbreaks shared allelic diversity and were distinct from US-1 and modern aggressive US lineages. US and EU famine era populations shared allelic diversity with lineages found in South America. The evolutionary relationship of historic and recent US and global lineages will be discussed in the context of possible pathogen migration routes and ancestry.

#### Taxonomic investigations warrants re-description of *Phytophthora* species infesting black pepper in India

M. Anandaraj\*, R. Suseela Bhai, Santhosh J. Eapen, R. Praveena, Bharath Nair, Cissin Jose and O.B. Rosana

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The foot rot disease of black pepper, *Piper nigrum* L., is caused by *Phytophthora* species. In cropping systems comprising of coconut, arecanut, cocoa, rubber, black pepper and cardamom, which are prevalent in states like Kerala and Karnataka of India, *Phytophthora* is of common

occurrence. There is a high possibility of one infected host serving as an alternative host to the other and thus helping the perpetuation and spread of this disease. The foot rot pathogen of black pepper was originally identified as *P. palmivora* by various workers. Because of its morphological dissimilarities with the typical *P. palmivora*, it was named *P. palmivora* MF4 in the revised tabular key by Stamps *et al.* in 1990. Subsequently, based on its morphology and biochemical profile *P. palmivora* MF4 was placed into a revised version of *P. capsici*. However, some other workers have designated those attacking *Piper* as *P. capsici* f. sp. *piperis* or as *P. tropicalis*.

Recently a wide range of *Phytophthora* isolates infesting black pepper were collected from different geographic regions of India and have been characterized and classified morphologically as well as phylogenetically. Comparative studies on colony morphology, sporangial ontogeny, sporangial morphology, mating and physiological studies of *Phytophthora* isolates from black pepper revealed prevalence of two groups – *P. capsici* and *P. tropicalis* with wide variations within the group. The isolates showed clear differences in colony morphology and sporangial types. Majority of isolates (95%) formed sporangia in water with long, unbranched or loosely branched, sympodial sporangiophores, whereas umbellate sporangial ontogeny was also observed in the rest of the isolates. Differences were also observed in sporangial shapes of the isolates with ovoid, elongate, globose, elliptical, ovoid obpyriform and distorted sporangia. All of them were A1 mating type and produced oogonia with amphigynous antheridia when paired with A2 mating types. Oogonia were usually round with smooth walls and antheridia mainly globose. Aplerotic and plerotic with thick wall oospores were also observed. Nearly half of the isolates (47%) produced chlamydospores. Invariably the optimum temperature for growth for all isolates was observed to be 25-30°C with the maximum temperature  $\geq 35^\circ\text{C}$ , a character typical of *P. capsici*. However, half of the isolates vary from *P. capsici* in their non-caducous nature, sporangial length to diameter ratio of more than 1.8 and medium to long pedicel length. rRNA internal transcribed spacer (ITS)-based phylogeny classify majority of the isolates belonging to clade 2, except a few isolates. However, a multi-locus sequence typing (MLST) involving nuclear and mitochondrial genes and whole genome sequencing (WGS) indicates the presence of a wide variety of genotypes with mixed characters indicating species diversification in black pepper agro-ecosystems. In this paper we describe the deviations of black pepper isolates from typical *P. capsici*/*P. tropicalis* based on their unique morphological, physiological and molecular features.

### ***PhytoWeb: A Database for Phytophthora Diseases of Horticultural Crops***

Santhosh J. Eapen, Alias Eldhose, S. Balaji, T.K. Vinod and M. Anandaraj\*

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*Phytophthora* is the most devastating pathogen of several horticultural crops causing enormous economic damage. The correct and timely identification of diseases is the basis for integrated



management of this dreaded pathogen. In this study an attempt has been made to develop a comprehensive database compiling information on various *Phytophthora* diseases of horticultural crops. The main objective of this work is to provide a common gateway to the researchers and institutions who have a general interest on *Phytophthora*. *PhytoWeb* contains information on 36 horticultural crops, 61 *Phytophthora* species with extensive details and 81 diseases that includes symptoms, epidemiology and management. Around 1,050 literature related to *Phytophthora* are also included for quick reference. PhyCC is another database integrated with *PhytoWeb* and contains detailed information on around 400 cultures maintained in the National Repository of *Phytophthora* at Indian Institute of Spices Research (IISR). A tool for identifying various species in the genus *Phytophthora* based on some key features such as asexual characters, host specificity, plant part, location and mating type is also made available. The database is available online (URL: <http://220.227.138.213/phytofura/phytoweb/index.php>), and can serve as an information resource for *Phytophthora* researchers worldwide. The database can be of great benefit to those who cannot afford the services of an expert pathologist, who have little or no information about the *Phytophthora* symptoms and host-specificity.



**Session: 3****Population biology and gene flow****New tools to understand evolving populations of *Phytophthora infestans* on regional, national and international scales for improved late blight management**

Cooke D.E.L.<sup>\*</sup>, Kessel G.J.T.<sup>2</sup>, Lees A.K.<sup>1</sup>, Lassen P.<sup>3</sup>, Baby S.<sup>3</sup>, Hansen J.G.<sup>3</sup>

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Late blight of potato, tomato and other solanaceous crops is caused by the destructive pathogen, *Phytophthora infestans*. Management relies on a combination of varietal resistance, minimisation of primary inoculum sources and correctly timed fungicide applications. The success of such management is however influenced by changes in the pathogen population. Studies on the population structure have highlighted a trend for major population shifts with widespread dispersal of damaging clonal lineages across some regions but highly diverse sexually recombining populations in other centres of potato production. Tracking of pathogen population change relies on standardised molecular tools to study pathogen diversity and a means of objectively comparing data collected in different locations. Over the 2013 and 2014 seasons an industry sponsored EU-wide monitoring project has generated data on >2200 late blight samples. The benefits of the European database ([www.euroblight.net](http://www.euroblight.net)) and the linked population genetic analysis tool 'Poppr', allowing a rapid web-based data analysis, will be presented. Using such tools we are exploring large datasets in unprecedented detail to improve our understanding of pathogen evolution and inoculum spread. For example, we are tracking the diversification of several thousand isolates of the 13\_A2 clonal lineage that has spread across Europe and to other continents from the local population first reported in Germany and the Netherlands in 2004. The knowledge gained from this data supports growers, breeders, advisors and the agrochemical industry. Expansion of this approach in collaboration with researchers in North and South America and Asia will also be discussed.

**Towards understanding the extent of genetic diversity of *Phytophthora* spp. infecting rubber (*Hevea brasiliensis*) and deploying molecular markers for resistance breeding**

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The rubber tree *Hevea brasiliensis*, producing 98% of the world's natural rubber is indigenous to the tropical rain forests of Central and South America. It is the only major commercial source of natural rubber and is one of the most recently domesticated crop species in the world. The unfavourable climatic conditions adversely affect growth and yield of rubber plants and also



act as predisposing factors for various fungal diseases. Of the many leaf diseases affecting rubber plants, abnormal leaf fall (ALF) caused by *Phytophthora* spp. is the most economically significant one in India causing shoot rot on immature rubber trees and abnormal leaf fall, patch canker and black stripe on mature rubber trees. The impacts of *Phytophthora* diseases on rubber production are reduction in latex yield (up to 32 per cent) and a reduction in growth due to leaf fall. ALF is an annually recurring disease of rubber in India and is reported to be caused by various species of *Phytophthora* including *P. meadii*, *P. botryosa*, *P. capsici*, *P. citrophthora*, *P. palmivora* and *P. nicotianae* of which *P. meadii* is the most prevalent one.

Population genetic studies performed with isolates of *Phytophthora* spp., collected over time and space, revealed existence of both A1 and A2 mating types in the population with the majority being A2 mating type. Genetic variability assessed through DNA-based amplified fragment length polymorphism (AFLP) fingerprinting technique indicated that all the isolates possessed unique AFLP profiles. The presence of mixed mating-type in the populations and the extent of AFLP diversity with no clonal genotypes, indicate that *Phytophthora* spp. affecting rubber reproduces both sexually and asexually during its life cycle enhancing genetic diversity within the population.

A genus-wide phylogeny of four *Phytophthora* species namely *P. meadii*, *P. botryosa*, *P. colocasiae* and *P. citrophthora*, reported to be prevalent in rubber plantations of India, was constructed using DNA barcode data. Since the use of individual loci have limitation in their ability to address the phylogeny, two nuclear DNA regions (Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA and the microtubule constituent protein  $\beta$ -tubulin) and one mitochondrial gene (cytochrome oxidase II) were used in the study to understand overall evolutionary history of the genus *Phytophthora*. Sequence information suggested that *P. meadii* has a hybrid origin with *P. colocasiae* as a parent and it is also possible that *P. citrophthora* is an asexual derivative of *P. colocasiae*.

Development of molecular markers linked to the resistance loci is essential for resistance breeding facilitating marker assisted selection in rubber. Allelic diversity based on simple sequence repeat (SSR) polymorphisms was studied at the locus encoding  $\beta$ -1,3-glucanase, a PR-2 protein having antifungal properties against *Phytophthora* causing ALF disease in rubber. An SSR marker (*hglu*) based on the repeat sequences, existing in the intronic region of  $\beta$ -1,3-glucanase gene was developed in rubber, which appeared to be highly polymorphic. Seven alleles could be detected among the cultivated popular clones of rubber and consecutively 16 allelic combinations/genotypes were identified. Allele mining for  $\beta$ -1,3-glucanase gene in wild *Hevea* accessions, derived from Acre, Rondonia and Mato Grosso provinces of Brazil, revealed the existence of 14 alleles forming 40 genotypes. Association between observed allelic diversity and functional variability of the trait (tolerance) is being assessed to identify the most potential allelic combination conferring resistance to ALF. Clustering was performed to group *Hevea* clones based on *hglu* allelic profiles/genotypes for establishing trait relationships. A putative RAPD marker linked to the loci conferring resistance to ALF disease in rubber was also identified and characterized at the nucleotide level. Association of this marker was noticed in wild accessions showing tolerance to *Phytophthora*.

To get insights into the functional diversity of the genes involved in disease resistance as well as to enhance the genomic resources for resistance breeding for *Phytophthora* tolerance, transcriptome sequencing was performed with RRIM 600, a *Phytophthora* susceptible cultivated clone and FX 516, an interspecific tolerant hybrid clone in both controlled and challenged conditions. Data analysis and wet lab confirmation of the digital gene expression are in progress.

### **Population structure of *Phytophthora infestans* infecting potato and tomato in India**

Nirmal B. J. Kumar<sup>1</sup> and P. Chowdappa<sup>2\*</sup>

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Late blight, caused by *Phytophthora infestans*, has emerged as the most destructive disease of potato and tomato worldwide. Severe outbreaks of late blight was recorded in India since 2008. A total of 276 isolates of *Phytophthora infestans*, 146 isolates from potato and 130 from tomato, were collected from major potato and tomato growing regions of India between 2009 and 2014. All the isolates were characterized based on aggressiveness, *in vitro* metalaxyl sensitivity, mating type, mitochondrial DNA haplotypes, SSR and RG57 DNA finger printing. All the isolates were highly aggressive, A2 mating type and resistant to metalaxyl. Majority of tomato isolates (127) and few potato isolates (14) were mtDNA haplotype IR<sub>1</sub> while most of the potato isolates (132) and 3 isolates from tomato were haplotype IR<sub>2</sub>. RG57 and SSR fingerprints were identical to the 13\_A2 clonal lineage reported in Europe. Variations of the D13 and SSR4 loci were designated as 13\_A2\_1, 13\_A2\_3a, 13\_A2\_3b and 13\_A2\_3c. This genotype has replaced the old population in India within three years of its first appearance in south west India. As all the isolates were metalaxyl resistant, management strategies has to be revised to combat this new population that has replaced the US-1 population of *P. infestans* in India. This study emphasise the importance of bio security in agricultural trade.

### **Genetic structure, aggressiveness and fungicidal sensitivity of *Phytophthora* associated with foliar blights of hot pepper**

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Chilli (*Capsicum annuum* L.) is one of the most widely cultivated vegetable crops worldwide, consumed in various forms such as fresh, dry powder, paste, and sauce. India is the world's single largest producer and exporter of chillies and its cultivation is mainly concentrated in



Andhra Pradesh, Karnataka, Maharashtra, Gujarat, Tamil Nadu, and Orissa. During the June-to-January cropping season of 2011 to 2014, severe foliar blight epidemics were observed in different states of India. Among the 82 *Phytophthora* isolates, recovered from blight-affected leaf tissues of hot pepper from different localities of India between 2011 to 2014, 63 isolates were identified as *P. boehmeriae* and 19 isolates as *P. capsici*, based on morphology, a similarity search of internal transcribed spacer sequences at GenBank, polymerase chain reaction (PCR) restriction fragment length polymorphism patterns, and species specific PCR using PC1/PC2 and PB1/PB2 primer pairs. *P. boehmeriae* and *P. capsici* isolates also exhibited characteristic protein and isoenzyme profiles. All isolates of *P. boehmeriae* were metalaxyl sensitive while *P. capsici* isolates were intermediate in sensitivity. *P. boehmeriae* isolates were highly aggressive and produced significantly ( $P < 0.01$ ) larger lesion than those of *P. capsici* isolates. Among 30 germplasms, none of the germplasms were found to be resistant. Thus, emergence of *P. boehmeriae* was responsible for severe leaf blight epidemics on hot pepper in South India, although it is not serious pathogen on any crop in any part of the world. These results have epidemiological and management implications.

### **Seasonal population fluctuation of *Phytophthora nicotianae* var. *parasitica* in Kinnow mandarin orchards under Punjab conditions**

Sarbjeet Kaur\*, Umesh Kumar Dhakad and S.K. Thind<sup>1</sup>

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*Phytophthora* diseases of Kinnow mandarin create serious threat in production and are responsible for ruin and decline of orchards in Punjab. Survival propagules of *Phytophthora nicotianae* var. *parasitica* present in nurseries and orchards are the main source of infection and spread. The prevalence of *Phytophthora* diseases in the districts of Fazilka (10-100%), Bathinda (10-50%) and Hoshiarpur (10-50 %) has been reported. The population dynamics of pathogen under Punjab conditions were determined. Soil samples were collected along feeder roots during May 2013 to April 2014 from the Kinnow mandarin orchards at three locations viz. Abohar, Hoshiarpur and Ludhiana. Soil plating method was adopted to determine the propagules per cc of soil on selective PARPH-CMA media. Significantly higher pathogen population (342, 402 and 354 propagules/cc of soil) was recorded in the month of July in Abohar, Hoshiarpur and Ludhiana respectively as compared to other months. Similarly, lowest population (7, 8 and 8 propagules/cc of soil) was recorded in these locations in June. The pathogen population showed an increasing trend from June onward and reached maximum in the month of July (366 propagules/cc of soil) and thereafter it showed decreasing trend. Recovery of pathogen population showed the positive correlation with mean temperature, rainfall and relative humidity.

## Genetic diversity of *Phytophthora infestans* isolates on Tomato and Potato from Eastern India

T. Dey<sup>1</sup>, S. Tewari<sup>2</sup>, K.L. Myers<sup>3</sup>, S. Tripathi<sup>4</sup>, W.E. Fry<sup>3</sup> and S. Guha Roy<sup>1\*</sup>

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Genetic diversity of *P. infestans* isolates from tomato and potato was assessed from Eastern and NE India as late blight becomes epiphytotic almost every alternate year and yet the information on the nature of *P. infestans* populations from these regions is fragmented and very limited. 60 isolates from farmers' field were collected from the states of Assam, Bihar, Meghalaya and West Bengal corresponding to the potato and tomato growing regions in these Eastern and NE parts of India. Genetic diversity was assessed based on phenotypic characterization which included mating type, sensitivity to fungicide metalaxyl, germination temperature ranges and aggressiveness on both hosts. Genetic characterization included mitochondrial haplotype and clonal lineage determination using RG57 probes, as well as using multiplexed SSRs. Effector diversity of the isolates were also evaluated. Data regarding these *P. infestans* populations will be discussed.



**Session: 4****Genetics and genomics****Genome evolution in filamentous plant pathogens***Sophien Kamoun*

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Many species of fungi and oomycetes are plant pathogens of great economic importance. To date the genomes of more than 30 of these filamentous plant pathogens have been sequenced, revealing remarkable diversity in genome size and architecture. Whereas the genomes of many parasites and bacterial symbionts have been reduced over time, the genomes of several lineages of filamentous plant pathogens have been shaped by repeat-driven expansions. In these lineages, the genes encoding proteins involved in host interactions are frequently polymorphic and reside within repeat-rich regions of the genome. This talk will review the properties of these adaptable genome regions and the mechanisms underlying their plasticity. I will also provide an update on our work on genome evolution in the lineage of the Irish potato famine organism *Phytophthora infestans*. Many plant pathogen species, including those in the *P. infestans* lineage, evolve by host jumps followed by adaptation and specialization on distinct hosts. However, the extent to which host jumps and host specialization impact genome evolution remains largely unknown. We and our collaborators resequenced several representative genomes of four sister species of *P. infestans*. This work revealed extremely uneven evolutionary rates across different parts of these pathogen genomes (a two-speed genome). Genes in low density and repeat-rich regions show markedly higher rates of copy number variation, presence/absence polymorphisms, and positive selection. These loci are also highly enriched in genes induced *in planta*, such as disease effectors, implicating host adaptation in genome evolution. These results demonstrate that highly dynamic genome compartments enriched in non-coding sequences underpin rapid gene evolution following host jumps.

**Comparative genomics for identification of orthology and phyletic patterns in *Phytophthora* sp.***Monica Chandran, O.B. Rosana, Santhosh J. Eapen and M. Anandaraj\**

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*Phytophthora* is a soil-borne plant pathogen which causes fruit, crown, and root rot in a wide range of plants. It causes foot rot disease in black pepper which is prevalent in all the pepper growing countries. In the current study, the entire secretomes of two *Phytophthora* isolates (05-06 & 98-93) infecting black pepper were compared with the secretomes of other *Phytophthora* species



such as *Phytophthora sojae*, *Phytophthora ramorum*, *Phytophthora infestans* and *Phytophthora parasitica* to identify orthologous and paralog genes that confer evolution. Mining the genomes of oomycetes through an evolutionary and comparative genomics approach, we identified and analyzed 17,301 secretory proteins, which revealed 1,771 protein families conserved in the selected genomes of *Phytophthora* species and 6,556 singleton proteins that include proteins specific to each species. Functional annotation of the corresponding secretory proteins revealed a relative abundance of parasite-specific effectors and transcription factors. This may point to important and specific regulators of genes involved in parasitism. Incorporation of orthology based phylogenetic profile will be a valuable approach for uncovering evolution due to adaptive pressure and genotype-phenotype relations of organisms in different geographical areas.

### **Species- specific detection of *Phytophthora* spp. infecting coconut, cocoa and arecanut crops using normal multiplex PCR and HRM analysis**

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*Phytophthora* infection is a major threat to most of the plantation crops in India and poses high economic loss to the farmers. Many species such as *Phytophthora palmivora*, *P. nicotianae*, *P. capsici*, *P. meadii*, *P. citrophthora* and *P. colocasiae* are either involved directly in causing the disease or are present in the plantation crops ecosystem. It is very difficult to identify these *Phytophthora* spp. using morphological characters. In this study, specific and sensitive PCR assay were developed for rapid detection of these six *Phytophthora* spp. infecting plantation crops. Initially *Phytophthora* samples were collected from five Indian states and were amplified using primers for ITS region and sequenced. Based on the sequences, species-specific primers were designed. When multiplex PCR assay was carried using these primers, it was found that *P. palmivora*, *P. nicotianae* and *P. capsici* could be easily identified based on the band size, but the other three species, viz., *P. meadii*, *P. citrophthora* and *P. colocasiae* gave approximately same band sizes. In order to differentiate these three species, High Resolution Melt curve analysis (HRM) was used. HRM primers were designed considering A/T allele difference and the three species could be differentiated by HRM-dissociation curves.

### **Comparative genome analysis of Irish Famine pathogen with Indian *Phytophthora infestans* isolate**

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Late blight disease caused by the oomycete, *Phytophthora infestans*, is the decimator of potato



cultivation costing over \$12 billion worldwide in crop losses and control measures. Yield losses due to late blight in India vary from year to year and range from 20-75%. The pathogen has been the subject of intense research pursuit due to its global economic importance. Management of this devastating pathogen is challenged by its remarkable speed of adaptation to control strategies such as genetically resistant cultivars. Keeping this in view, re-sequencing the whole genome of Indian *P. infestans* isolate was conducted at CPRI, Shimla. Genome size of this pathogen is about 240 Mb. We had generated ~20X coverage of whole genome sequence of *P. infestans* (S15) using Ion Torrent (Next Generation Sequencing) technology. Total of 501913 raw reads were generated having average read length of 150 bases. The assembly of these reads yields 218 Mb data of *P. infestans* having largest contig length is 80,989 bp and average contig size of 431. Presently targeted re-annotation of novel genes especially effector genes is in progress using comparative genomics approach where the *P. infestans* S15 novel genes are compared with sequence of *P. infestans* strain T30-4, which has been sequenced by Broad Institute, USA. We are going to present the comparative analysis at genome as well at gene level between the *P. infestans* strain T30-4 genome and Indian isolate *P. infestans* S15 genome. The results demonstrate the potential of comparative genome analysis for exploring the effector proteins and its pathogenesis, which would help in designing the fruitful management strategies against late blight disease.

### **An efficient method for zoospore production, infection and real-time quantification of *Phytophthora cajani* causing *Phytophthora* blight disease in pigeonpea**

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Phytophthora blight caused by *Phytophthora cajani* is an emerging disease of pigeonpea (*Cajanus cajan* L.) affecting the crop irrespective of cropping system, cultivar grown and soil types. Current detection and identification methods for *Phytophthora* species rely primarily on cultural and morphological characteristics, the assessment of which is time-consuming and not always suitable. Sensitive and reliable methods for isolation, identification, zoospore production and estimating infection severity are therefore desirable in case of *Phytophthora* blight of pigeonpea. In this study, protocols for isolation and identification of *Phytophthora* blight of pigeonpea were standardized. Also the method for zoospore production and *in planta* infection of *P. cajani* was developed. Quantification of fungal colonization by *P. cajani* using real-time PCR was further standardized. *Phytophthora* species infecting pigeonpea was identified based on mycological characters such as growth pattern, mycelium structure and sporangial morphology of the isolates and confirmed through molecular characterization (Acc. No. KJ622200 - KJ622208; KJ010534 - KJ010538). For *Phytophthora* disease development, zoospore suspension of  $1 \times 10^5$  zoospores per ml was found optimum. *Phytophthora* specific real-time PCR assay was developed using specific primers based on internal transcribed spacer (ITS) 1 and 2. Use of real-time PCR allowed the quantitative estimation of fungal biomass in plant tissues. Detection sensitivities

were within the range of 0.001 pg fungal DNA. A study to see the effect of elevated CO<sub>2</sub> on *Phytophthora* blight incidence was also conducted which indicated no significant difference in disease incidence, but incubation period delayed under elevated CO<sub>2</sub> as compared to ambient level. The zoospore infection method for *Phytophthora* blight of pigeonpea will facilitate the small and large scale inoculation experiments and thus devise a platform for rapid and reliable screening against *Phytophthora* blight disease of pigeonpea. qPCR allowed a reliable detection and quantification of *P. cajani* in samples with low pathogen densities. This can be useful in early warning systems prior to potential devastating outbreak of the disease.

### **Improved Genome assembly of *Phytophthora ramorum* by third generation sequencing technology**

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*Phytophthora ramorum* is the causal agent for Sudden Oak Death disease that wiped out most of the Oak trees in coastal California. In 2006, the draft genome of *P. ramorum* was sequenced along with *Phytophthora sojae* at approximately 10X coverage. The genome was assembled to 65 MB, 2576 scaffolds with 12 MB gaps. Recently we have undertaken PacBio sequencing for improving the assembly statistics. While the PacBio technology generates one to two orders of magnitude longer sequence reads than Sanger sequencing, its high error rate hampers effective assembly. The total PacBio reads generated from our sequencing efforts were 435399 with a maximum read length of 28625 base pairs and average read length of 5000 base pairs. Error correction of PacBio reads is performed using ECTools with the previously published assembly as reference. This resulted in 147429 error corrected PacBio reads that were further used in downstream assembly process. In addition, two sets of Illumina libraries were used for hybrid assembly e.g; libraries from genome isolate ( 62922216 reads and 75 bp average read length) and libraries from clonal isolate (52837176 reads with an average read length 100 bp). We have used several assemblers alone or in combinations for optimizing the assembly outcome. The best result so far has been obtained with PacBio error corrected reads with 7589 unitigs (contigs without gaps) from previous assembly and Illumina short reads. We first assembled the error corrected PacBio reads using Celera assembler followed by merging celera assembly with unitigs from previously published assembly using Minimus. This was followed by another assembly including the Illumina short reads and the Minimus output using SSPACE and SSPACE-Long Reads. The final hybrid assembly now has 78,285,078 base pairs on 1114 scaffolds with an N50 value of 130116. There is an improvement in the assembly only with 450621 gaps, where the previous assembly had total gaps of 12227865. CEGMA analysis on the genome reveals presence of 95.56% (partial), 91.94% (full) eukaryotic COGS. Further work is in progress for gene prediction using RNAseq data as training dataset. This will enable us analysing and understanding pathogenicity of *P. ramorum* in better accuracy.



## Session 5

### Pathogenesis and plant innate immunity

#### From genomics to effectors: how understanding oomycete biology has contributed to disease control

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Oomycetes cause a wide array of destructive diseases of plants and animals. Many of these pathogens use secreted proteins, called effectors, that can enter inside host cells to subdue the immune systems of their intended hosts. Oomycetes are fungus-like organisms that belong to the kingdom Stramenopila, which includes photosynthetic algae such as diatoms and kelp. Oomycete plant pathogens, including *Phytophthora* and *Pythium* species and many downy mildews, cause billions of dollars of damage to crops, forestry and ornamental plantings each year. We have generated draft genome sequences for numerous oomycete plant pathogens. These genomes encode a family of effectors, the RXLR effectors, that is particularly large (130-1200 per genome) and rapidly evolving. The effector proteins enter host cells by binding a cell surface lipid, a property that they share with effectors from some fungal pathogens of plants and humans.

We have used transcriptional profiling to identify *P. sojae* effector genes that play an active role in promoting infection. Combining this information with mathematical modeling and high throughput functional screens for plant defense suppression has revealed an elite subset of effectors comprising around 10% of the repertoire that appears to be responsible for most of the contribution of this family to virulence and host immune suppression. Many family members are individually indispensable for virulence. The information obtained from studying effectors is enabling us to develop new technologies for preventing disease

#### Transcriptome assisted label-free proteomic analysis of *Phytophthora capsici* X *Piper nigrum* L. phytopathosystem.

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Black pepper (*Piper nigrum* L) is a spice crop well known for its culinary and medicinal uses worldwide. Production of black pepper has significantly been affected by *Phytophthora capsici*. In this study, we applied transcriptome assisted label-free quantitative mass spectrometry to identify novel molecular components regulated during early host immune response of black pepper leaves when challenged with *P. capsici* 24 hours post inoculation. Functional annotation of the identified components was carried out using BLAST2GO tool which lead to identifying

novel proteins and genes from black pepper. A total of 532 novel proteins were identified and annotated in the present study. Of these, 194 proteins were differentially regulated between control and test protein dataset. Major differential expression took place in a number of pathways underlying the response of black pepper to *P. capsici* such as photosynthesis, protein processing in endoplasmic reticulum, carbon metabolism, oxidative phosphorylation and endocytosis. Some of the significantly down-regulated protein components have roles in carbohydrate metabolic process, response to stress, single-organism transport, transmembrane transport, response to stimulus and single-organism localization, as identified by Revigo tool. The gene expression data were also consistent with the quantitative proteomics results. Our novel approach sheds light on the hitherto unknown molecular networks that are involved in defense of black pepper against *Phytophthora* infection and provide a platform for future studies.

### **Incompatible Interactions of *Piper colubrinum* with *Phytophthora capsici*: Insights from gene expression studies**

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Foot rot caused by *Phytophthora capsici* is a devastating disease of black pepper (*Piper nigrum* L.), the most important spice crop of India. *Piper colubrinum* L., a distant relative of black pepper, highly resistant to *Phytophthora capsici* was used for host-pathogen interaction studies. Transcriptome characterization of the plants challenge inoculated with *Phytophthora capsici* identified genes involved in pathogen recognition and signalling, anti-microbial proteins and transcription factors besides NBS – LRR type resistance genes. The *de-novo* assembled transcripts were analysed for microRNAs and their corresponding mRNA targets. *Piper*-specific novel miRNAs were also identified from the study. Presence of effector genes of *P. capsici* in the interactive transcriptome were also analysed. Gene expression studies using quantitative RT-PCR revealed differential accumulation of resistance gene (R gene) transcripts of *Piper colubrinum* upon inoculation with different strains of *Phytophthora capsici*. Defence related genes like osmotin, beta-1,3-glucanase and thaumatin like protein were found to be expressed at higher levels during 72 hours post inoculation (hpi) at 900, 54 and 450 folds respectively. Transcription factors bHLH, MYC, MYB and WRKY showed peak expression during early hours of pathogen inoculation. *In planta* expression studies on glucanase inhibitor gene from *P. capsici* and beta 1-3 glucanase gene from *Piper colubrinum* indicated a gene to gene interaction resulting in the suppression of *Phytophthora*. Genes for cell wall degrading enzymes and other pathogenicity genes like Pectate lyase and RXLR genes from *P. capsici* showed peak expression at early hours of challenge inoculation compared to genes like glucoside hydrolase and NPP which expressed at later periods.





## ***Medicago truncatula* genes implied in Mycorrhiza and *Phytophthora palmivora* infection identified through genome-wide association studies**

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Plants constantly engage in interactions with both, pathogenic and symbiotic microbes. The Mediterranean legume model plant *Medicago truncatula* enables us to study both types of interactions as it is host to nitrogen fixing bacteria, arbuscular mycorrhiza fungi and the root pathogen oomycete *Phytophthora palmivora*. Furthermore, more than 190 fully genotyped *M. truncatula* accessions permit genome wide association studies (GWAS) to identify genetic loci impacting on plant-microbe interactions. In this study we surveyed 170 accessions for their response to seedling root infection by *P. palmivora*. Subsequently, we used GWAS to identify loci contributing to seedling length as well as disease symptom extent. Interestingly, we found that some genes at candidate loci showed mycorrhiza-colonisation specific changes in transcription suggesting that they might impact on this symbiosis. Knockdown of two *M. truncatula* candidate transcripts in transgenic hairy roots resulted in enhanced resistance to *P. palmivora* colonisation on the root surface. Most recent data on their further characterisation will be presented.

## **Towards understanding the black pepper-*Phytophthora* pathosystem - using integrated transcriptome and proteome datasets**

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*Phytophthora* foot rot is a devastating disease in Black pepper which is caused by *Phytophthora capsici*. The molecular aspect of pathogenesis and defence response by the pathogen and by the plant is still not fully understood. Use of genetic resistance is an important and major component in the integrated management of this disease. To understand molecular mechanisms of host-pathogen interaction and resistance in black pepper, gene and protein expression studies on a moderately resistant variety IISR Shakthi was investigated by using quantitative proteomics, transcriptomics and differential expression studies. Quantitative expression proteomics from control and infected plant samples yielded spectrum of proteins from major metabolic pathways, defense and signal transduction regulated upon inoculation with *P. capsici*. Strong up regulation of group of enzymes including cysteine proteinase. Subtilisin like protease and other lipid transfer protein groups were observed in Shakthi. Kinases, zinc finger, germin like proteins, WRKY and ethylene responsive transcription factors were also found to be up regulated. For understanding on role of R genes we explored the interactive transcriptome of IISR Shakthi developed using Illumina platform, annotated with Blast 2Go software and five R gene loci were selected for from real time expression analysis. The aligned transcripts of these loci showed characteristic domains of R genes. The dynamics of transcript abundance in moderately resistant



(IISR-Shakthi) and susceptible (Subhakara) genotypes upon challenge inoculation with virulent isolate (05-06) from 0 to 72 hrs after inoculation was studied by real time expression analysis. Resistance Loci 6113 and 26441 were found to be upregulated during early hours in IISR Shakthi whereas it was down regulated in susceptible genotype. Loci 81,753 and 26441 were down regulated in Shakthi, whereas susceptible variety recorded the up regulation at early hours. The finding of 2 prominent up regulated R genes against *P. capsici* indicates its role and utility in resistance. RACE mediated full length isolation of R gene (loci 6113) from both these genotypes yielded sequences corresponding to important NB-ARC domain that has major role in regulating the activity of the R proteins. The variation in the domain would possibly be used in the development of gene specific markers for resistance breeding in black pepper.

### **Nodal inoculation - a quick and easy inoculation technique for *Phytophthora* blight of Pigeonpea**

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Phytophthora blight (PB) of Pigeonpea [*Cajanus cajan*(L.) Millsp.] caused by *Phytophthora cajani* is potentially third important disease. The PB is emerging as a potential threat to pigeonpea production, especially during excessive rain within a short span of time that create temporary flooding coupled with hot and humid weather. Ridge sowing and seed treatment with metalaxyl is recommended against PB. However, it is not adopted widely due to unavailability of ridge maker and metalaxyl formulation for seed treatment. Resistant cultivar is most feasible technology delivered through seed that do not require any additional efforts from the farmers. Selection of resistance from the germplasm, advanced crossing materials or through mutation is possible only with appropriate screening technique. Currently available screening techniques are either lacking to infect the plant or gives inconsistent result. The new nodal inoculation technique is able to induce the disease from germination to 50 days old plants and first node to 8<sup>th</sup> node. This technique is based on the removal of petiole by pulling it up and inoculation of mycelia disc of *P. cajani* on the node. Relative humidity above 85% and temperature 25- 30 °C favored the infection. Plants less than twenty days old were highly susceptible with >95% node infection and most of the infected plant died within 5 days of inoculation. The susceptibility of node decreased with the increasing age of plants from 20 to 50 days old plant. The disease reaction varied from restricted lesions around the node with survival of plants to extended lesions and killed the plants. Nodal inoculation technique differentiated plant from resistant to susceptible group. Adopting nodal inoculation method, a trained technician can inoculate 90-100 plant/ hr.

**Session: 6****Epidemiology and decision support systems****Epidemiology and Decision Support Systems**

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Significant improvements in forecasting plant diseases have been made possible by the remarkable advances in computing power and by our enhanced understanding of the population biology of plant pathogens. Advances in computing power mean that it is now possible: to obtain high resolution weather data in near real-time; to obtain high resolution (over time and space) weather forecasts; and to operate complex simulation models of plant diseases very rapidly in on-line sessions. Enhanced understanding of the population genetics of plant pathogens enables us to determine if the population of a particular plant pathogen is complex or simple (little diversity). In the case of very simple pathogen populations (dominated by one or a few clonal lineages), it is feasible to determine phenotype of the major lineages, and to use this information in conjunction with other relevant data to inform management decisions.

To illustrate these possibilities a Decision Support System (DSS) developed to aid the management of potato or tomato late blight in the USA will be described. The DSS has been named “BlightPro”(Small et al. 2015 ), and initial evaluations indicate significant potential to enhance the efficiency of fungicide use and to reduce the variance associated with disease suppression(Small et al. 2015). The DSS obtains historical weather data and weather forecasts from the Northeast Regional Climate Center, hosted at Cornell University and which is associated with the National Weather Service in the USA. The actual weather data come from the weather station of choice of the user, which may be an on-farm weather station, or the nearest weather station that is on-line. The weather forecast (on a 2.5 km grid) comes from the national weather service and the forecast is updated several times per day. These data are used to drive either of two late blight forecasts (Blitcast or Simcast). Additionally the system hosts the late blight simulation model Lateblight 2004 (Andrade-Piedra et al. 2005), and the user can use this model to evaluate the effects of diverse management strategies. The system contains host resistance data on more than 50 potato cultivars and more than 40 tomato cultivars, so that host resistance can be incorporated into the Simcast forecast.

Currently (2011-2015) in the USA, a consortium of investigators report occurrences of late blight on a public website (<http://blight.eas.cornell.edu/blight/>). The genotype of *P. infestans* from many of these samples is determined and also reported on the website. The phenotype of these genotypes for several important characteristics is known (Danies et al. 2013) and this information can then be used to inform specific management decisions.

## Indoblighcast– A simple generic DSS for Late blight management

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Late blight is the most dreaded disease of potato worldwide including India. The disease progression rate of *Phytophthora infestans* on potato is very fast, which necessitates forecasting the time of disease appearance, hence, the importance of forecasting of late blight appearance. World over many models have been developed for late blight forecasting using meteorological data. However, the available models are empirical and need local calibration which is the major bottleneck in the wide spread use of forecasting models for practical application. Similar to most late blight forecasting models available worldwide a forecasting model for Indian conditions called “JHULSACAST” has been developed and calibrated for different locations in India viz., Punjab (Jalandhar), Western Uttar Pradesh (Modipuram), Tarai region (Pantnagar) and plains of West Bengal (Kalyani). This model uses daily thermohygrograph data to derive the daily accumulated hourly temperature and relative humidity values which greatly limits the adoption of the model across regions. Therefore, a simple generic Late Blight forecasting system was developed and incorporated as a DSS using the data of late blight appearance dates and meteorological conditions at different AICRP (Potato) centres over the past several years. The model requires daily minimum & maximum temperature and minimum & maximum relative humidity from meteorological observatory as inputs. Using the temperature data the Physiological days (P-days) is calculated every day. The daily maximum and minimum relative humidity is interpolated to the night time temperature to derive the night time relative humidity and both P days and night time relative humidity are accumulated over the previous 7 days. Test of various permutations and combinations of accumulated P-days and accumulated relative humidity showed that the thumb rule “if the accumulated P-days over 7 days exceeds 52.5 and accumulated RH over the same period exceeds 525 for 7 consecutive days, then late blight would appear within the next 15 days” gave reliable forecasts across agroecologies. Statistical comparisons of observed and predicted dates of late blight appearance showed that the mean absolute error was 10.48 while the residual mean square error was only 13.17. The Willmott D index was 0.84 which is quite close to unity thus indicating high accuracy of model predictions. Receiver Operating Characteristics (ROC) analysis also confirmed the superiority of this combination (accuracy of 76.32%, AUC of 0.725) to predict late blight appearance as well as its non appearance in unfavourable years. This model has been validated using historical data as well as on real time basis for the past two years at several locations both in the hills and plains.

As regards the DSS, it consists of two modules one for data entry and the other for the general users to see the status of late blight forecast. The data entry module consists of a database for authorised personnel to “Add data” (to enter data), “Load data” (for viewing already entered data), “Edit data” (to change entered data values) and “Delete data” (to remove data) on daily basis. The other module is for checking the late blight appearance status. This consists of a window with drop down menu to select location which would lead to another window with a



calendar, a run model button and a 2 x 15 dimensional array for display of disease severity status. The user can select a date in the calendar and click on the “Run model” button which would then display the status of late blight for fifteen days prior to the selected date in the 2 x 15 dimensional arrays. The first row displays the date while the second row displays the disease severity values and the late blight status in colour code. The colour code consists of three colours; Green colour (for disease severity values up to 4) indicates that late blight is not likely to appear soon; yellow colour (for disease severity values between 4-6) indicates that late blight would appear very soon; and red colour (for disease severity values above 6) indicates that the weather conditions have become suitable for late blight and it can appear any time within fifteen days. Thus, depending upon the time required for taking control measures, the user may start preventive measures at yellow colour indication. The display of the status of late blight of the previous 15 days enables the user in decision making because if late blight appearance had been predicted earlier and preventive sprays had been given, then it would help to decide whether the spray is to be repeated or not. Thus, the structure of the DSS enables its use not only to forecast late blight appearance but also decide on the need based application of fungicides.

### **Forecasting of late blight in India- A success story**

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Late blight caused by *Phytophthora infestans* is one of the most dreaded diseases of potato worldwide and causes significant loss in production. Relationship between *P. infestans* and the weather is well understood and has been utilized for developing disease forecasting models across the globe including India. Ambient temperature, RH, light, fogginess, rainfall, dew, wind velocity etc. have a strong relationship with the blight pathogen and the disease. Work on late blight forecasting in India started in 1950's when Chaudhuri and Pal utilized rainfall data together with the dates of appearance of late blight in Darjeeling hills for 12 years using moving graph concept of Cook's and Hyre's. They demonstrated that 7-day moving graph with critical rainfall of 1.8 inch could accurately predict the appearance of late blight in that area. The criteria for late blight forecasting however, differed from for different Indian hills. Bhattacharyya and coworkers in 1982 utilized daily weather data including temperature, rainfall and RH and the date of actual appearance of late blight for Shimla, Shillong and Ootacamund. They established that if a 7 day moving precipitation of at least 30 mm for Shimla, 28.9 mm for Ooty and 38.5 mm for Shillong hills within a mean temperature of 23.9 °C or less continue for 7 consecutive days, late blight would appear within 3 week and further if hourly temperature between 10-20°C was associated with RH ≥80 % for continuous 18 hr for at least 2 consecutive days, late blight would appear within a week. This model has been put to successful use for predicting late blight since 1983 and it is still working very well especially in Shimla hills. Similarly, late blight forecasting for eastern plains has been developed based on blight favourable days. The model specifies that blight favourable period of two consecutive days comprising of average minimum temperature

(7-15°C), average maximum temperature of 22-25°C, RH >75% and average sunshine < 5hr per day would predict late blight within 7-21 days of satisfying the conditions. Singh and coworkers in 2000 used moving graph concept and developed a computerized forecasting model 'JHULSACAST' for western UP for both rainy and non rainy years. For rainy years, if measurable rains was 0.1-0.5 mm for a minimum of two consecutive days, 5-day moving > 85% RH period  $\geq 50$  hrs, and 5-day moving congenial temperature 7.2-26.6°C  $\geq 105$  hrs, blight would appear within 10 days. For non-rainy years, if 7-day moving > 85% RH period was  $\geq 60$  hrs and 7-day moving congenial temperature period between 7.2-26.6°C was  $\geq 120$  hrs, blight would appear within 10 days. These models have been validated and are able to predict late blight accurately in western Uttar Pradesh. This model was further calibrated by Arora and coworkers for predicting appearance of late blight in Punjab under non-rainy conditions. The model specifies that a 7-day moving sum of RH  $\geq 85\%$  for at least 90 hr coupled with a 7-day moving sum of temperature between 7.2-26.6°C for at least 115 hr would predict appearance of late blight within 10 days of satisfying these conditions. JHULSACAST model has also been calibrated for Tarai Region of Uttarakhand and the plains of West Bengal. These blight forecasting models have enabled us to predict appearance of late blight in different regions in India well in advance. Based on such forecasting models the necessary alerts are issued through press and radio and the potato farmers are advised to start a relevant fungicide spray schedule for that. Late blight forecasting in India is helping the farmers to use the fungicides in an effective and in a rational manner avoiding their unnecessary use and thereby managing the disease in a cost effective and environmental friendly manner.

### **Global potential distribution of *Phytophthora infestans* under current and climate change situations**

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A simulation model was developed using CLIMEX to assess the global potential distribution of *Phytophthora infestans* for current and future climate change situations. *P. infestans* is considered a devastating pathogen infecting tomato and potato causing significant economic loss. Late blight caused by *P. infestans* can completely destroy the above-ground parts of plants (stems, leaves, tomato fruits) and can also affect potato tubers. The current study is undertaken to generate potential distribution maps as information on the effect of climate change on the distribution of *P. infestans* is scarce. Compare Location function of CLIMEX was used to assess the distribution of the pathogen. Currently the pathogen is distributed in most parts of South America, Asia, Australia, Africa, North America and Europe. Under climate change situation with 1<sup>o</sup> and 2<sup>o</sup>C rise in temperature, shift in the favourable/unfavourable regions to the pathogen





is predicted in different countries. In India, north-western and central part is expected to be less conducive for the establishment of the pathogen under projected climate change scenarios. The present findings were also compared with DIVA- GIS (bioclimatic modelling software) and a similar distribution pattern was observed for *P. infestans*. Validation of the model was carried out for selected locations with reference to its seasonal incidence.

### **Survey and surveillance of *Phytophthora* black pod rot of cocoa in southern transition zone of Karnataka**

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*Phytophthora palmivora* is one of the major constraints in cocoa production causes significant pod losses up to 30% and killing up to 10% of the trees annually in Karnataka state of India. *P. palmivora* is responsible for seedling blight in nursery; leaf blight and black pod rot in field inflicting heavy crop losses during rainy season. Therefore, an intensive roving survey was conducted during 2014-15 to know the incidence and severity of the disease in nursery and fields of cocoa growing areas of Southern Transition Zone of Karnataka. The propagules of the pathogen cause lesions on leaves and pods which results in pod rot. Survey on the disease in the nursery and field showed that the extent of disease affecting crop quality and quantity of pods in different locations which was widespread particularly in rainy season. Highest severity of Seedling blight was observed in raised bed nurseries compare to poly bag nurseries in Shivamogga district. Further the highest incidence and severity of black pod rot disease was recorded in Shivamogga (28.50%), Thirthalli (27.00 %) and Sagara (22.00%) taluks of Shivamogga district followed by Udupi, Chikkamanglore and Dakshina Kannada districts due to continuous rain fall or high moisture conditions and the crop was grown as intercrop with arecanut is vulnerable for the attack of pathogen due to the presence of pathogenic variability.



**Session 7:****Host plant resistance, molecular breeding and variety development****Potato late blight in developing countries, intensity, management and future risks***Gregory A. Forbes*

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Potato late blight (PLB) caused by *Phytophthora infestans* is one of the most costly of all plant diseases. Estimates due to both crop loss and fungicide use go as high as 15 billion USD per year. PLB is virtually ubiquitous, occurring almost everywhere that potato is produced, but its intensity is variable depending principally on climatic factors. The disease is primarily managed with frequent fungicide applications, and the role of host plant resistance has been considered negligible. However, some recent analyses would indicate that host resistance is playing an important role in some parts of the developing world, which gives hope for the potential of this disease management strategy. Use of host resistance to reduce risk of crop loss and modulate fungicide dependency may be particularly promising in areas where cultivar turnover is still occurring. To date, the greatest source of risk to farmers has been greater disease pressure due to pathogen evolution toward increased aggressiveness, and/or fungicide resistance. Risk due to climate change would appear to be highly variable, with disease intensity increasing in some areas and decreasing in others. Nonetheless, in some regions increasing temperatures will move PLB into areas where it was previously not problematic and this may lead to important changes in production practices.

***Piper colubrinum*, resistant root stock as an option for eco-friendly disease management of *Phytophthora* foot rot of black pepper (*Piper nigrum*) caused by *Phytophthora capsici* Leonian***Y.R. Sarma*<sup>1</sup>, *K. Nirmal Babu*<sup>2</sup>, *L.N. Hegde*<sup>3</sup>, *Homey Cheriyan*<sup>4</sup> and *P. Jayaraj*<sup>5</sup><sup>1</sup>FAO, Expert, Former Director, Indian Institute of Spices Research, Kozhikode, Kerala, India<sup>2</sup>AICRPon Spices, Indian Institute of Spices Research, Kozhikode, Kerala, India<sup>3</sup>AICRPS Center, University of Horticultural Sciences, Bhagalkot, Karnataka, India<sup>4</sup>Directorate of Arecanut and Spices Development, Kozhikode, Kerala, India<sup>5</sup>Krishi Vigyan Kendra, Kerala Agricultural University, Kannur, Kerala, India

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*Phytophthora* foot rot still remains as a major production constraint in black pepper cultivation. At present it is a nightmare to pepper farmers all over the globe where black pepper is grown. Slow decline associated with root infection caused by *Radopholus similis* and *Meloidogyne incognita*, the plant parasitic nematodes, combined with malnutrition is the other malady affecting black Pepper. Efforts by all the member countries of International Pepper Community (IPC), Jakarta,



consisting of Brazil, India, Indonesia, Malaysia, Sri Lanka and Vietnam resulted in developing Integrated Disease management (IPM/IDM) module to be practiced in all the IPC member countries. Disease resistant to foot rot and Slow decline of black pepper are not available at present. Black pepper (*Piper nigrum* L), originated from Western Ghats of India and spread to all pepper growing countries. The striking uniformity of disease incidence in all pepper growing countries indicates its common origin and *Phytophthora capsici* is the predominant pathogen all over. All parts of black pepper are prone to *P. capsici*. There are areas where the foliar infection is major (foliar phase of the disease), and there are areas with severe root/ collar infections (Soil phase of the disease) which are fatal and there are regions where mixed infections also occur. With the amount of biodiversity of black pepper in India, it is generally expected the host resistance for *P. capsici* might be available in the region. But the intensive efforts made so far by R&D institutions in India, did not yield high degree of host resistance to these devastating pathogens and pests. Efforts to develop resistant varieties still remained elusive. But five varieties viz IISR-Shakti and IISR-Thevam developed at Indian Institute of Spices Research (IISR) and Panniyur-8 and Vijaya, developed at Kerala Agricultural university (KAU) and Ademane pepper developed at University of Horticultural Sciences (UAS) were relatively field tolerant but not really high yielding. *Piper colubrinum*, a South American distant species of *Piper* was found to be a source of multiple resistance as it is resistant *P. capsici*, *R. similis* and *M. incognita*. *P. colubrinum* is a diploid (2n: 26), while cultivated black pepper is a tetraploid (2n: 52). But researchers from Kerala Agricultural University (KAU) reported the development of an interspecific hybrid (*P. nigrum* X *P. colubrinum*) which showed resistance to *Phytophthora* but the yields were very low. This was attributed to low pollen fertility. Further studies are in progress to study the inheritance pattern for resistance to these pathogens and its adaptability.

The multiple resistance of *P. colubrinum*, though proven, could not be effectively utilized as root stock through grafting and was not practiced in pepper growing countries. This was probably due to earlier reported graft incompatibility because of anatomical reasons. However recent observations on cleft grafting of black pepper with improved varieties especially Panniyur 1 scions on *P. colubrinum* root stock has been found highly successful and is being practiced by some enterprising farmers both in Kerala and Karnataka. Absence of complete graft union is noticed in several cases. Though the graft union is not uniform due to differential growth of scion and root stock (the growth rate of black pepper being faster), most of the grafted vines were found performing giving reasonable yields even after 12 plus years. The reported yield of 5-12 yr old vines ranged from 1-3kg of dry pepper/per vine. More information needs to be generated to ascertain the sustainability, productivity, of these grafts for their longevity and studies are in progress. Based on the field observations at present, grafted pepper vines gave economic returns up to the age of 12 years, especially in regions which are hot spots for *Phytophthora*. This clearly indicated the potential of this technology as a major option for foot rot management. *P. colubrinum* produces suckers regularly, and *in situ* grafting can be undertaken in the field directly, with time. However *P. colubrinum* is highly sensitive to water stress and maintenance

of good soil moisture is absolutely essential to maintain the root stock. High mortality of the grafts was reported earlier in the absence of soil moisture.

The root and collar infection( soil phase) of the disease which is very crucial for its sustainability of the crop, can be avoided from infection. In view of this technology, large scale use of fungicides required for soil application, can be avoided and consequently reduce the possible environmental pollution. But foliage (leaves, stems and spikes)remains highly vulnerable to infection by *P. capsici*. In areas where foliar infection is serious, need based prophylactic sprays with fungicides is imperative to check foliar infections. Thus this technology is ecofriendly and ecologically sustainable in foot rot management in black pepper. This technology is highly relevant in Areca-Pepper mixed cropping system where irrigation is a practice to maintain high soil moisture. This may also by default , give protection from nematode damage .

Realizing the importance, Directorate of Arecanut and Spices Development (DASD) along with Krishi Vigyan Kendra (KVK) Kannur of KAU, under Mission for Integrated Development of Horticulture (MIDH), Govt. of India, are popularizing this grafting technology. AICRP Spices, Sirsi Center of University of Horticultural Sciences, Bhagalkot, Karnataka in collaboration with Directorate of Horticulture, Govt. of Karnataka is also involved in encouraging the farmers in Karnataka. About 300 thousand grafts are being produced in Sirsi region of Karnataka and is becoming popular in Kerala too.

### **Crop resistance improvement by mining natural and induced variation in host accessibility to *Phytophthora palmivora***

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Increasing crop yield to feed the world is a grand challenge of the 21st century but it is hampered by diseases caused by filamentous plant pathogens. The arms race between pathogen and plant demands constant adjustment of crop germ plasm to tackle emerging pathogen races with new virulence features. To date, most crop disease resistance has relied on race specific disease resistance genes that are effective only against a subset of pathogen isolates. Our hypothesis is that disease resistance based on manipulation of host accessibility processes has a higher probability for durability, and is best identified using a broad host-range pathogen. We are employing the filamentous pathogen *Phytophthora palmivora* to mine plant alleles and unravel host processes providing microbial access in roots and leaves of monocot and dicot plants. We utilize plant symbiosis mutants and are using genetic variation to elucidate general mechanisms of colonization by filamentous microbes in plant roots and shoots. Our systems of choice are economically relevant crops such as barley and wheat to allow immediate translation into breeding programs. In addition, we challenge our findings by testing beneficial fungi to assess commonalities and differences between mutualist and pathogen colonization.



## **Effect of plant age and cultivar on population of *Phytophthora nicotianae* var. *parasitica* causing foot rot/gummosis in Kinnow mandarin and Sweet orange**

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Foot rot/gummosis caused by *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse has become an important factor in the decline of Kinnow mandarin, Sweet orange and nurseries in the Punjab state. The malady poses a serious threat to the future of potential citriculture in the state and reduces the life expectancy, quality and yield potential of the trees. It survives by the mean of propagules in soil. In present studies, pathogen propagule dynamics were studied in relation to plant age and cultivar. 5 and 10 year old Kinnow mandarin plant and Kinnow mandarin and sweet orange cultivar were selected at Fazilka location. Studies were conducted during June 2013 to December 2013. Soil collected from feeder root area of disease affected plants was plated on selective PARPH-corn meal agar media. Studies revealed that 10 year old plants had more propagules/cc of soil in comparison with 5 year old plant. Population was maximum i.e. 371 and 325 propagules/cc of soil in the month of July for 10 year and 5 year old plant respectively. Feeder root rot index was relatively higher for 10 year old plants. Kinnow mandarin and Sweet orange cultivar did not differ significantly for pathogen population and feeder root rot. However, the pathogen population recorded higher on Sweet orange cultivar.

## **Identification and *in silico* characterisation of putative resistance genes in black pepper and related species**

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Sequence based homology approach using conserved nucleotide binding site domains of plant resistance genes was used to identify resistance gene analogs from black pepper and two related *Piper* species. Cloning and sequencing primed by degenerate oligonucleotides identified 53 resistance gene analogs from *Piper nigrum*, *Piper colubrinum* and *Piper ornatum* and were named as *Piper* resistance gene analogs (*Piper* RGA). The majority of the plant resistance genes belong to NBS-LRR family which is involved in recognition during pathogen invasion and defense signaling. NBS-LRR class of resistance proteins is found to be effective against biotrophic and hemibiotrophic pathogens. The deduced amino acid sequences of the *Piper* RGAs detected the presence of consensus domains such as NB-ARC (nucleotide-binding adaptor shared by APAF-1, certain *R* gene products and CED-4) with P-loop, Kinase-2a and GLPL motifs which are characteristics of plant *R* genes. Sequence analysis grouped *Piper* RGAs into non-toll interleukin

receptor (non-TIR) subfamily of NBS-LRR based on the presence of tryptophan as final aminoacid residue of Kinase-2 domain. Phylogenetic analysis clustered *Piper* RGAs with non-TIR R gene analogs of different plant species. The frequency of non-synonymous to synonymous substitution (Ka/Ks) ratio indicated that resistance gene analogs from *P. colubrinum* and *P. ornatum* are under purifying selection due to their narrow genetic diversity as only clonally propagated materials were introduced in India. Resistance gene analogs of *P. nigrum* are under diversifying selection revealing substantial sequence diversity. The identified *Piper* RGAs were compared with *P. nigrum* and *P. colubrinum* transcriptome database available at IISR website to identify probable candidates expressed during *Phytophthora* infection. *In silico* analysis revealed the similarity of 53 *Piper* RGA to 249 R gene sequences from *P. nigrum* transcriptome and 176 R gene sequences from *P. colubrinum* transcriptome having characteristic domains of NBS-LRR R gene family. Hence these *Piper* RGAs are valuable resources for discovering R genes and improving disease resistance in black pepper against *Phytophthora* infection.

### Identification of sources of resistance against *Phytophthora* in arecanut

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Arecanut (*Areca catechu* L.) is a commercially important plantation crop predominantly grown in humid tropics of India. Productivity of the palm is affected by a number of diseases and nutritional disorders. Among the fungal diseases, *Phytophthora* diseases cause major economic loss every year. *Phytophthora meadii* is a major pathogen of this palm being the causal agent of three major diseases viz., fruit rot, bud rot and crown rot. Fruit rot disease inflicted a yield loss of 50-90%. Prophylactic spraying of 1 % Bordeaux mixture has been recommended for the control of these diseases. While, Bordeaux mixture preparation and spraying during rainy season is a cumbersome process and also causes copper toxicity to grazing animals. Host-plant resistance is considered as eco-friendly, economical and sustainable option to mitigate arecanut yield losses due to *Phytophthora* diseases. Identification of stable sources of resistance to fruit rot disease is a prerequisite to develop resistant variety. Seven arecanut varieties (Mangala, Sumangala, Sreemangala, Swarnamangala, Mohitnagar, Nalbari, Srivardhana), dwarf mutant (Hirehalli dwarf) and two wild types (*Areca triandra* and *Areca concinna*) were screened against *Phytophthora meadii* under *in vitro* condition by following detached arecanut method. All seven arecanut varieties and a dwarf mutant were infected and showed typical symptoms of fruit rot disease three days after inoculation while the wild types, *Areca triandra* and *Areca concinna* were not infected and showed resistance reaction to *P. meadii*. Thus, these resistance sources could be exploited to develop fruit rot disease resistant arecanut hybrids through hybridization and biotechnological approaches.





## Managing late blight disease of potato through introgression of RB gene in Indian potato cultivars

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Late blight of potato caused by *Phytophthora infestans* is still the single most important impediment to potato cultivation globally. Worldwide potato crop losses due to late blight are estimated at € 12 billion per annum. Presently the disease is managed through cultural practices, fungicides and host resistance. Of these, management of late blight through host resistance will remain the most environmentally and economically preferred option globally despite the fact that none of the variety could sustain the blight onslaught for more than 5-7 years. Besides conventional and marker assisted breeding, efforts are on to integrate resistance genes in commercial cultivars of potato through transgenic/molecular approach. The R gene responsible for race non-specific, broad-spectrum resistance in *Solanum bulbocastanum* has been cloned by two independent groups in USA (RB) and The Netherlands (*Rpi-blb1*) which conferred resistance to a wide range of isolates of *P. infestans*. In this study the RB-transgenic elite Katahdin line SP951 used for introgression breeding was obtained from University of Wisconsin, Madison, USA through the collaboration of India - USAID - ABSP II. In our breeding program, RB transgenic Katahdin line SP951 was used to introgress the RB based resistance in two Indian commercial potato cultivars Kufri Jyoti and Kufri Bahar through conventional breeding. The F<sub>1</sub> hybrids were characterized molecularly for the integration of RB gene through PCR using MAMA primers and expression analysis of RB and SGT transcript were assessed by qRT-PCR. The hybrids of Kufri Jyoti x SP951 were superior over hybrids of Kufri Bahar x SP951 in terms of late blight resistance. On the basis of resistance level, tuber characters and other agronomic traits we have selected five Kufri Jyoti hybrids (KJ-16, KJ-21, KJ-65, KJ66, and KJ-77) for temperate highlands. The late blight resistance of the hybrid clones was evaluated in confined green house under controlled environmental conditions as well as under confined field trials against most complex races of *P. infestans* prevalent in the country. Southern hybridization and expression of RB and its defence regulatory SGT gene through Real Time PCR supplemented the bioefficacy of the selected hybrids. Flanking sequence analysis for the integration of T-DNA region encoding RB cassette was confirmed in the genome of promised transgenic hybrid clones. Microarray analysis of promising hybrid lines revealed activation of R and R- like genes which might have involved in imparting resistance against late blight.



## Session 8

# Disease management- fungicide and biological control

### Management of *Phytophthora* diseases in the tropics

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*Phytophthora palmivora* has several hundred recorded hosts and causes global yield losses to cocoa production of 20-30% and tree deaths of up to 10% annually. Other hosts, including oil palm, coconut, rubber, papaya, durian, black pepper and citrus suffer similar losses to this, and related, species. Integrated management of *Phytophthora* diseases depends on disrupting the disease cycle, and should be incorporated into an integrated crop management program. Options available to farmers include the thorough preparation of the soil before planting to increase the activity of antagonistic soil microbes, selection of disease-resistant genotypes when they are available, weed management, pruning to manage the crop and shade canopy, mulching, water and nutrient management, regular, complete harvesting, sanitation and hygiene and the use of fungicides. We have found that the adoption of IPDM technologies is greatly improved if farmers participate in the identification of research priorities, are involved in the research and extension process and are presented with a series of graded options rather than a set of fixed recommendations. We have also found that improved farm management has a greater impact on alleviating poverty and improving livelihoods when integrated with health and education training programs.

### Management of *Phytophthora* blight (*Phytophthora capsici*) of peppers

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*Phytophthora* blight, caused by *Phytophthora capsici*, is an important disease of peppers. Crop losses of 100% by *P. capsici* have been recorded in commercial pepper fields in Illinois. *P. capsici* can infect pepper plants at all growth stages, causing seedling death, root rot, crown rot, stem blight, leaf spot, and fruit rot. In some cultivars, only fruit are infected. Four approaches were evaluated for management of *Phytophthora* blight in peppers, which included: (i) using resistant cultivars, (ii) induction of resistance in plants by red-light treatment, (iii) crop rotation, and (iv) fungicide application. To identify resistant pepper cultivars to *P. capsici*, accessions/cultivars of bell pepper were tested in the greenhouse and field, and several cultivars were found resistant/tolerant to *P. capsici*. Pepper seedlings grown under red light (600-700 nm) for four weeks reduced *P. capsici*-infection by 74% in the greenhouse. To establish effective crop rotation for management of *P. capsici*, host-range of the pathogen was determined and survival



of the pathogen in soil was investigated. A 4-year crop rotation with nonhost plants and effective weed control are recommended for management of *Phytophthora* blight of peppers. More than 50 fungicides with potential effects were evaluated for their efficacy for control of *P. capsici*. Ametoctradin + dimethomorph (Zampro 525F), captan (Maestro 80DF), cyazofamid (Ranman 400SC), dimethomorph (Forum 4.16 SC), famoxadone + cymoxanil (Tanos 50WDG), fluazinam (Omega 500F), fluopicolide (Presidio 4SC), mandipropamid (Revus 2.09SC), and mefenoxam (Ridomil Gold Copper 65WP, Ridomil Gold EC 4SC) fungicides were effective in controlling *P. capsici* in pepper.

### **The 30 year journey: Integrated *Phytophthora* root rot management in avocados**

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*Phytophthora* root rot (PRR), caused by *Phytophthora cinnamomi* (Pc), is the most severe constraint to orchard productivity in the Australian avocado industry. The success of the industry today is attributed to the targeted research, development and widespread adoption of integrated management options for the PRR, and relentless multi-media communication to growers. Current best practice relies on 1) planting *Phytophthora*-free trees sourced from accredited nurseries 2) careful site selection and preparation, including adequate soil water drainage to reduce the duration of free soil water which is required for long periods of infection 3) mulching and/or composts to improve soil structure, encourage root regeneration and stimulate microbial activity to reduce the survival of Pc propagules 4) Selection of rootstocks which are tolerant to Pc, for example Dusa and a new selection SHSR-04, 5) Optimal tree nutrition, including calcium amendment to suppress Pc, 6) Judicious use of phosphorous acid and metalaxyl and 7) Providing practical information and training to avocado growers (technology transfer). These areas will be discussed in relation to our group's research efforts over several years, and novel approaches we are currently trialing will be presented.

### **In search for new targets to control *Phytophthora* pathogens**

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*Phytophthora infestans*, the Irish famine pathogen, is the causal agent of late blight in potato and tomato. This oomycete has a hemibiotrophic life style, a narrow host range and a large genome of ~ 240 Mb. Comparative genomics revealed features illuminating its success as a pathogen, such as rapid turnover and massive expansion of families encoding secreted proteins, and peculiar gene innovations resulting in proteins with oomycete-specific domain combinations. Examples of novel proteins are the GPCR-PIPKs (GKs) and GPCR-INPPs (GIs) that have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with either

a phosphatidylinositolphosphate kinase (PIPK) or inositol polyphosphate phosphatase (INPP) domain at the C-terminus. This domain structure suggests that GKs and GIs use GPCRs to directly feed extracellular signals into phospholipid signalling pathways. For one GK we could demonstrate a role in asexual development, including spore germination, hyphal elongation and sporangia cleavage, whereas inactivation of another GK disturbs sexual development. We have also indications for a role of GK in the dynamics of the actin cytoskeleton. This is a dynamic but well organized intracellular framework that is indispensable for the viability of eukaryotic cells. Its functions range from intracellular transport, formation of contractile rings, nuclear segregation, and endocytosis. Microscopic analysis of *P. infestans* transformants expressing the actin binding peptide Lifeact-eGFP revealed actin filament cables and plaques. The latter are nearly immobile structures with average lifetimes exceeding one hour; much longer (over 500-fold) than the lifetimes of actin patches in fungi. Moreover, in contrast to actin patches in yeast, plaque disassembly is not accompanied with formation and internalization of endocytic vesicles. In conclusion, the oomycete specific features of GKs, GIs and actin plaques justify more in depth research to evaluate their potential as target for novel oomycides.

### ***Trichoderma* based eco-friendly management of *Phytophthora* diseases**

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Plant diseases caused by *Phytophthora* spp. are very crucial yield determinants in several horticultural crops. Use of antagonist such as *Trichoderma* is being explored for the management of many of the diseases. The primary inoculum of *Phytophthora* spp. survives in soil utilizing the plant debris as a source of food. The secondary spread is through zoospores. Hence, the use of antagonists as seed treatment and soil application will help in managing the disease spread through the primary inoculum. For eco-friendly and sustainable management of the disease, four antagonists, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma viride* and *T. hamatum*, were explored worldwide under *in vitro* and *in vivo* conditions. *In vitro* screening with bio-antagonists effective against soil borne pathogens is a simplistic approach to understand a biological system in disease control. Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. In last three decades, a lot of researches have been carried out on the antagonistic nature of several species of genus *Trichoderma* (Papavizas, 1985; Chet, 1987) which had shown highest potential against soilborne fungal pathogens. Both *T. harzianum* and *T. viride* showed highest potential against many soilborne fungal pathogens. Researches on *T. harzianum* and *T. viride* as a biocontrol agent also showed differential antagonistic potential among isolates (D'Souza *et al.*, 2001; Mohanty, 2003). The different *Trichoderma* spp. isolates demonstrated variable mycoparasitic activities which had been worked out by various research groups. The biocontrol potential of *Trichoderma viride*-ES1 and *Pseudomonas fluorescens*-Bak15 was worked out against potato late blight pathogen and was known to reduce the disease severity and found that the foliar application of *T. viride*-ES1 has good potential in controlling the late



blight disease of potato (Zegeye *et al.*, 2011). The effect of talc based formulations of *T. harzianum* isolates was seen to reduce *P. capsici* infection in red pepper (Sriramet *et al.*, 2009) and the potential use of ISR eliciting isolates was discussed. Several research groups aimed to investigate antagonistic activity of *Trichoderma* spp isolates against *P. nicotianae* (Singh and Islam, 2010) and found that *T. harzianum* (0034H) was found highly inhibitory to *P. nicotianae* in dual culture followed by *T. harzianum* (0034M) and *T. harzianum* (0034W). *T. viride* (0034S) showed least effective to inhibit the mycelial growth of *P. nicotianae*. In another study *Trichoderma* spp. showed some promise against isolate of *Phytophthora parasitica*. It and found that isolates T4 and T7 were highly effective against the above pathogen under *in vitro* conditions (Datta *et al.*, 2011). *In vitro* antagonism showed that *P. parasitica* significantly inhibited by *T. harzianum* and *T. virens* (84.96%) in citrus (Gade, 2012). Sharma *et al.* (2014) reviewed many of the *Trichoderma* spp. as effective agents against *Phytophthora* infection in (Citrus, Cauliflower, Chilli). *T. viride* and *T. harzianum* showed 77.77% antagonism against *Phytophthora colocasiae* (Ambuse and Bhale, 2015). In this context and from the perspective of Integrated Pest Management (IPM), biological control is an additional method that can help in reducing the *Phytophthora* diseases to economically viable levels, with a concomitant decrease in the use of chemicals (Krauss and Soberanis, 2001; Bajwa and Kogan, 2004). Validation of effective strains of *Trichoderma* against different pathotypes spp. in different agroecosystems and their mechanism of action is still a research gap which needs to be explored in future.

### **Strategic management of root knot nematode induced *Phytophthora* disease complex in horticultural crops through biopesticides**

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Plant parasitic nematodes are important biotic constraints in diverse crops affecting world food production. Among the phytoparasitic nematodes, root knot nematodes (*Meloidogyne* spp.) are one of the most devastating pests affecting a large number of crops and causing a negative impact on their health, yield and quality. Vegetables are the most preferred hosts for root knot nematodes. These nematodes are not only pathogenic by themselves, but also predispose the wounded roots to easy entry of other soil borne pathogenic fungi and bacteria which aggravate the disease severity and yield loss. The resulting 'nematode disease complex' situation also results in breakdown of disease resistance in healthy plants and leads to wilting and ultimately death of plants. Root knot nematodes interact synergistically with large numbers of root infecting fungi like *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium*, *Pythium*, *Macrophomina* etc. in the rhizosphere of different crops. Indeterminate use of chemical pesticides is not only expensive but also causes serious threat to biotic ecosystems and environment. Hence, the use of biological control agents plays a safe and effective role in sustainable management of nematodes in open fields and protected conditions. Indian Institute of Horticultural Research,

Bengaluru has developed effective biopesticide formulations and standardized integrated nematode management packages for mitigating nematode problems in horticultural crops. Seed treatment with biopesticides at 15 – 20 g/kg seeds, substrate treatment (in nurseries) with 10 g/kg of substrate with biopesticides and soil application of biopesticide enriched organic mixture (FYM/ vermicompost and neem cake) at 1 kg/m<sup>2</sup> with repeated application at 100 g/m<sup>2</sup> at regular interval of 30 days in standing crop has drastically reduced damage due to root knot nematodes and associated disease complexes in several horticultural crops like banana, tomato, okra, chillies, capsicum, cucurbits and gerbera. The biopesticide formulations can also be sprayed on the plants or given through drip/ drenching at 5g/lit. In gerbera, application of *Trichoderma harzianum* enriched vermicompost at 50 g/ m<sup>2</sup> recorded minimum incidence of *M. incognita* (root knot index -3.9) and *Phytophthora cryptogea* (19.2%) with 37.6% increase in yield compared to untreated control. In capsicum, application of *Pseudomonda fluorescens* as seed and substrate treatment followed by field application significantly reduced the root knot nematode population by 74% and also reduced the severity of *Phytophthora capsici*. It also increased the yield of bell pepper by 26% and the benefit cost ratio due to the additional returns accrued by application of biopesticides was 3.8.

### **Development of bio-control formulations for management of late blight of tomato (*Lycopersicon esculentum* Mill.)**

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Late blight incited by *Phytophthora infestans* is a destructive disease of tomato worldwide. The plant growth-promoting antagonists, which elicit ISR and enhance plant growth, are being used as safe alternatives to synthetic fungicides for the management of plant diseases. In this study biocontrol formulations were developed and evaluated which were found to be effective for less reduction in disease incidence and induction of systemic resistance in tomato against *P.infestans* in comparison with fungicides and also persuaded growth promotion. Seed treatment with Seedpro, a microbial growth promoter having a combination of *T. harzianum* OTPB3 and *B. subtilis* OTPB1 caused significant increase in growth parameters compared to *P. putida* OPf1, mancozeb and untreated control due to higher production of IAA and GA<sub>3</sub>. Reduction in the incidence of late blight was positively linked to increase of phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and β-1,3-glucanase, the defense-related enzymes in tomato seedlings treated with microbial consortium of OTPB3 and OTPB1 followed by foliar spray formulation of *P.putida* OPf1. The effects were on par with fenamidone and mancozeb treatments. The results revealed that seed treatment with Seedpro and foliar spray of *P.putida* OPf1 has practical significance in the management of late blight disease and also to produce healthy planting material and plant growth enhancement in tomato.





## **New vistas in management of *Phytophthora* in black pepper (*Piper nigrum* L.)**

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Black pepper is highly prone to infection by *Phytophthora* during monsoon season coinciding with heavy rainfall and high humidity which is congenial for the growth and development of *Phytophthora* that cause foot rot disease. As part of IDM, biocontrol is the most sustainable option. Recently promising results have been reported with endophytic fungi. They draw basic benefits from the host such as nourishment and physical protection and in return the host may be protected from biotic and abiotic stress by producing secondary metabolites like alkaloids, antibiotics or toxins. The present research work is highlighting their status as sources of biocontrol agents against black pepper *Phytophthora*. About 125 isolates of endophytic fungi were isolated from black pepper and evaluated against *P. capsici* *in vitro*. Nine isolates showed antagonism to *P. capsici* and plant parasitic nematode *Radopholus similis*. These isolates belong to species of *Diaporthe*, *Fusarium*, *Phomopsis*, *Annulohyphoxylon nitens*, *Daldinia eschscholzii* and *Ceriporia lacerata*. These are first report of its kind in black pepper. Similarly several strains of actinomycetes have been found to protect plants against diseases as source of agro active compounds, plant growth-promoters and biocontrol tools at the rhizosphere. Thirty rhizosphere actinomycetes isolated from different parts of Kerala and Karnataka were evaluated against *P. capsici* and shortlisted nine isolates having high antagonistic activity. The isolates are positive for IAA and siderophores production, which contribute to plant growth promotion and biocontrol. The dehydrogenase activity was also higher showing higher microbial metabolic activity. The Actinomycetes in consortia mode was found promising. The isolates were identified as *Streptomyces* sp. (IISRact5), *Streptomyces tauricus* (IISRact9) and *Kitasatospora setate* (IISR act2). The endophytic fungi and actinomycetes, therefore, are likely to be potential candidates for novel secondary metabolites which may be of importance for various PGP and biocontrol applications.

## **Organic management of taro leaf blight incidence caused by *Phytophthora colocasiae***

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Taro leaf blight (TLB) caused by *Phytophthora colocasiae* has become a major concern in all taro growing countries including India causing yield loss of 25-50% (Misra *et al.*, 2008). Present recommendation to manage the disease includes the application of metalaxyl /mancozeb. Most of the effective fungicides are synthetic, toxic and potentially polluting and organic production of taro is gaining importance. Managing plant diseases, insect pests and weeds are the greatest



challenges in organic farming. Compost and compost tea have been widely explored as an eco-friendly option for controlling diseases. Later it was found that vermicompost can also provide the same effect. At ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, India, the possibility of utilizing vermicompost and vermiwash to manage taro leaf blight incidence was explored. Thirty five vermicompost samples were collected from different parts of the country and assessed their microbial diversity and efficiency to control the target pathogen. Based on these characters, vermicompost produced at Kerala Agricultural University was selected for the study. Pot culture study was conducted for two years using the variety Sree Kiran with 14 treatments comprising soil application of vermicompost, seed treatment with vermiwash, spraying with vermiwash and combination of all these at different intervals. The treatment, soil application of vermicompost @700 g per plant in three splits + seed treatment with 10% vermiwash + spraying and drenching with 10% vermiwash at 60 & 90 DAP significantly reduced the disease incidence and increased the yield. Based on the results of pot culture study, the efficacy of the treatment was tested in field for two consecutive years along with fungicides recommended for taro leaf blight management. It was found that the above mentioned treatment increased yield (87.5%) and reduced TLB incidence (50%) compared to control plants. Same trend was observed in second year also and was comparable with the protection offered by metalaxyl- mancozeb. Hence, vermicompost and vermiwash can be used as an eco-friendly option for managing leaf blight in taro. The enzyme study conducted suggested that in addition to microbial action for disease suppression, concomitant stimulation of several defence mechanisms is a strong possibility.

### **Bio-efficacy of endophytic *Trichoderma* species against *Phytophthora palmivora* the causal agent of black pod disease of cocoa**

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*Phytophthora palmivora* is an oomycetus pathogen known to infect many plants grown in humid tropics and causes significant economic loss. Black pod disease of cocoa and bud rot disease of coconut are the important diseases caused by *P. palmivora* in India. Under natural conditions these pathogens are suppressed by many endophytic *Trichoderma* sp. In this study *Phytophthora palmivora* isolates infecting coconut and cocoa were collected from disease endemic areas and among the collected isolates three were identified as highly virulent (CPCRI-KA-286, CPCRI-AP-334 and CPCRI-KL-216) based on pathogenicity test. These isolates were used as test fungus to evaluate the bio-efficacy of endophytic fungus isolated from cocoa pods. Five endophytic *Trichoderma* isolates (CPCRI-COC-1, CPCRI-COC-2, CPCRI-COC-3, CPCRI-COC-4 and CPCRI-COC-5) obtained from cocoa pods were evaluated for antagonistic activity against three highly virulent *P. palmivora* isolates (CPCRI-KA-286, CPCRI-AP-334 and CPCRI-KL-216). Among five endophytic *Trichoderma* isolates tested against *P. palmivora* isolates, CPCRI-COC-3 isolate showed complete inhibition of growth of all the three highly virulent *P. palmivora* isolates within three days after inoculation, followed by CPCRI-COC-4.



## An efficient and eco-friendly tool for sustainable management of taro leaf blight (*Phytophthora colocasiae*)

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Taro, *Colocasia esculenta*, is grown under flooded or dry land conditions on over 1.8 million ha (4.5 million acres) and is the fourteenth most consumed vegetable worldwide. The leaves are eaten cooked, and the corm is baked, boiled, fried, pounded into a paste (poi), or made into flour. In Taro, *Phytophthora colocasiae*, the cause of leaf blight disease causes reductions in corm yield of 25-50% in India. The warm, humid days and cool, wet nights of the tropics are ideal for reproduction and spread of *P. colocasiae*. The disease can be managed to some extent with chemical combinations. But due to leaf texture, angle of leaf, wax coating on the leaf surface and coincidence of the disease incidence with monsoon rains the disease management has become a major challenge to farmers. Not only has that depending on chemicals alone led to resistance development in pathogen but also causes environmental pollution. The sustainable option for preventing or minimizing the outbreak of disease is with the proficient use of both biocontrol agents and chemicals. With this object, an experiment was conducted during *Kharif*, 2012-13 at Vegetable Research Station, ARI, Rajendranagar, Hyderabad. The experiment was laid by using taro var., Sathamuki which was susceptible to leaf blight disease with eight treatments (application of biocontrol agents such as *Trichoderma viride* and *Pseudomonas fluorescens* and chemicals mancozeb 0.2% and their combinations) in three replications in randomized block design. The data on per cent disease index was collected at 90 days after planting and data on yield (t ha<sup>-1</sup>) was recorded at the time of harvest. The results revealed that all the treatments were significant in reducing the disease incidence and increasing the yield over control. However the treatment T<sub>5</sub> (T<sub>1</sub> - Seed treatment (10 gm /kg seed) and soil application of *Trichoderma viride* (T1 isolate) @ 2 kg ha<sup>-1</sup> at the time of planting + Foliar application of mancozeb@ 2.0%) was significantly superior in reducing the disease incidence by 56 per cent over control with the yield of 28.3 t ha<sup>-1</sup> compared to other individual treatments.

## Studies on efficacy of fungicides and their dosage against *Phytophthora colocasiae* causing leaf blight of Taro

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Leaf blight caused by *Phytophthora colocasiae* is the most destructive disease affecting taro (*Colocasia esculenta*). The disease has become a major constraint for taro cultivation. It

causes heavy yield losses to the extent of 25-50% every year. Presently, fungicides remain as important strategy to manage leaf blight of taro. Many of these fungicides are extensively used and administration of these chemicals lack proper dosage. Therefore a preliminary study is inevitable before these fungicides are extensively used for the management of taro leaf blight. In view of this the present study was undertaken to evaluate the efficacy of various fungicides viz., Mancozeb, Pyrachlostrobin+Metiram, Tebuconazole, Cymoxanil, Metalaxyl, Copper oxychloride, Dimethomorph, Thiophanate methyl, Azoxystrobin, Bordeaux mixture on *in vitro* to determine the sensitivity of *P. colocasiae* to fungicides by poisoned food technique at recommended and half therecommended doses. The results of the study revealed that complete inhibition in growth of mycelium of *Phytophthora colocasiae* was recorded in Mancozeb(0.8g l<sup>-1</sup>), Pyrachlostrobin+Metiram(1.0 ml l<sup>-1</sup>) and Tebuconazole (1.0g l<sup>-1</sup>) at recommended concentration. However Cymoxanil (2.0g l<sup>-1</sup>), Metalaxyl(2.5 g l<sup>-1</sup>), Copper oxychloride (1.0ml l<sup>-1</sup>) significantly reduced the growth of mycelium by 98.0, 92.9 and 91.0 per cent respectively, whereas fungicides Dimethomorph(1.0g l<sup>-1</sup>) (89.5 per cent), Thiophanate methyl(0.5g l<sup>-1</sup>) (74.6 per cent), Azoxystrobin(3.0g l<sup>-1</sup>) (72.5 per cent) could also inhibit the mycelium of the pathogen. Minimum per cent inhibition of the pathogen was recorded in Bordeaux mixture(8.0g l<sup>-1</sup>) (70.3 per cent) even at recommended doses. Whereas at half therecommended doses Pyrachlostrobin+Metiram(0.5 ml l<sup>-1</sup>) and Tebuconazole (0.5 g l<sup>-1</sup>) shown complete inhibition of the growth of mycelium of *Phytophthora colocasiae*. Though 100 per cent inhibition of the pathogen was observed by Mancozeb at recommended dose has inhibited only 83.9 per cent at half the recommended concentration (0.4g l<sup>-1</sup>). Thiophanate methyl showed an inhibition of 62.2 per cent followed by Bordeaux mixture 61.4 per cent and azoxystrobin 58.5 per cent. The study revealed that the leaf blight of Taro can be managed with alternate use of Mancozeb(0.8 g l<sup>-1</sup>) or Pyrachlostrobin+Metiram(0.5 ml l<sup>-1</sup>) or Tebuconazole (0.5 g l<sup>-1</sup>) concentrations effectively and economically.

### **Fungicide sensitivity of *Phytophthora infestans* isolates on tomato and potato from Eastern India.**

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The potato and tomato growing regions of Eastern and NE India comprising of Assam, Bihar, Meghalaya and West Bengal are severely affected by late blight almost every alternate year. However, pathogen population based comparisons of the fungicide sensitivity of isolates from different genetic backgrounds is lacking. Therefore to assess the fungicide sensitivity of the *Phytophthora infestans* populations, 60 isolates of *P. infestans* both from tomato and potato were collected from these potato and tomato growing regions of Eastern and NE India. Sensitivity of the isolates to non-phenylamide commercial fungicides having different modes of action like the Dithiocarbamate- Mancozeb (Indofil M45®, Osho chemical Industries), Dimethomorph



(Acrobat®, BSF), Azoxystrobin (Amistar®, Syngenta) were evaluated *in-vitro* by poison agar technique. The results were analyzed and an attempt was made to correlate them with the population structure of *P. infestans* in these regions as revealed earlier through genotypic and phenotypic characterizations, which will be discussed.

### **Integrated management of black shank disease of FCV tobacco (*Nicotina tabaccum* L.) in filed and its effect on leaf yield and quality parameters.**

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The field experiment was conducted at zonal agricultural research station, Shimoga in replicated randomized blocks in highly susceptible variety FCV special to test integrated management schedules against black shank disease in FCV tobacco. Integration of press mud @ 250g/plant along with *Trichoderma harzianum* @ 25g/plant spreading at the time of planting gave significantly higher green leaf yield (8304 kg/ha), cured leaf yield (1382 kg/ha), total grade index (1018 kg/ha) and recorded least incidence of black shank disease (5.50 per cent) when compared to other treatments. Untreated check recorded the lowest green leaf yield (1475 kg/ha), cured leaf (249 kg/ha), total grade index (127 kg/ha) with highest disease incidence of 30.50 per cent. All the leaf quality constituents viz; nicotine (0.82 per cent), reducing sugars (20.53 per cent), chloride (0.2 per cent) were within the acceptable limits in the treatment combination of press mud and *Trichoderma harzianum* compared to other treatments.

### **Management of fruit rot of arecanut caused by *Phytophthora meadii* under *in vivo* conditions**

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The arecanut palm, *Areca catechu* L. is the source of the common masticatory nut, popularly known as arecanut. Arecanut is one of the most important commercial crops in the Southeast Asia. It is affected by many diseases among them fruit rot is dominating one. During the year 2014 bulk farm trials was conducted in six different locations of Karnataka viz.. Varadamula, Hosavi, Tuppooru, Kouti, Wodeyala and Bobbi village where rainfall was ranged from moderate to heavy. Different fungicides like Conventional Bordeaux mixture @1%, Stabilized Bordeaux mixture @1%, Ridomil MZ @0.2%, COC @0.3%, Biofight @0.5%, Biopot @0.5% and Curzate @0.2% were evaluated at different concentrations under field conditions to manage the fruit rot disease. The result indicated that the number of fallen nuts due to fruit rot incidence was ranged from 0.30 to 33.17 /palm. Application of conventional Bordeaux mixture @1% has significantly



reduced number of fallen nuts due to koleroga (fruit rot) of arecanut (0.91) and also attempt was made to record the yield which has significantly increased the nut yield (green nut 9230.44 kg/ac and dry nut 1200.32 kg/ac) at six different locations followed by Stabilized Bordeaux mixture at 1%.

### **Biological management of potato late blight disease using *Bacillus subtilis* var. *amyloliquefacians***

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*Phytophthora infestans* is one of the devastating Oomycetes fungus which causes late blight disease in potato. The disease causes severe yield loss up to 100 per cent under favourable conditions. A study was undertaken, in order to manage the disease using antagonistic bacterium, *Bacillus*. Twenty four antagonistic bacteria were isolated from various regions of Tamil Nadu. Along with these isolates, FZB 24 strain of *Bacillus subtilis* var. *amyloliquefacians* (M/s Novozymes South Asia Ltd.) was used to determine their efficacy *invitro* and *invivo* conditions. The FZB 24 strain showed a maximum of 35.6 per cent inhibition against the *P. infestans* *invitro*. Further, this was carried forward to the glasshouse and field trials with different dosages. In the glass house experiment, when the potato plants were sprayed with 0.2 per cent of *Bacillus subtilis* var. *amyloliquefacians* (FZB 24) six times at 7 days interval recorded a late blight disease reduction of around 50 per cent over control. Similarly, under field conditions the above treatment reduced the disease severity significantly and registered a higher tuber yield of 15.5t/ha as against 9.4t/ha in control plots. Hence, the *Bacillus* sp. can be exploited for the effective management of late blight disease of potato.

### **Evaluation of bioagents for managing *Meloidogyne incognita* and *Phytophthora* disease complex in capsicum (*Capsicum annuum* L. var. *grossum* Sendt) under protected conditions**

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Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt), also popularly called as capsicum or sweet pepper or shimla mirch is cultivated in most parts of the world for its pleasant flavour coupled with rich contents of vitamins and minerals. In India, capsicum is widely grown in polyhouses for higher productivity and round the year cultivation. However, continuous cultivation of the same crop, constant higher temperature and humidity and use of high agronomic inputs in protected cultivation provide conducive conditions for the introduction and rapid multiplication of nematode diseases, especially root knot nematodes (*Meloidogyne incognita*). It causes severe



root damage that ultimately leads to yield losses to a tune of 12 to 40% in capsicum. Nematode damage also predisposes the roots to easy entry of other soil borne pathogenic fungi and bacteria, thereby causing a disease complex which aggravates the disease intensity and in severe cases, leads to complete crop failure. Biological control agents hold a good promise for managing the nematode problems in an eco-friendly and sustainable manner.

Field experiments were conducted in 2013-14 and 2014-15 in capsicum (cv. Orobelle) at IIHR, Bengaluru under protected conditions to evaluate several biocontrol agents *viz.*, *Bacillus subtilis*, *B. pumilus*, *Paecilomyces lilacinus* and *Trichoderma harzianum* for the management of root knot nematodes and associated disease complexes in capsicum. The treatments were given singly and in combination as seed treatment (20g/kg seed), substrate treatment (10g/kg cocopeat) and soil application at 1 kg/m<sup>2</sup> after enrichment in FYM (2 kg of biopesticides in 1 t of FYM). The pooled analysis indicated that the plots treated with *Bacillus subtilis*, *Trichoderma harzianum* and *Paecilomyces lilacinus* as seed treatment, substrate treatment and soil application of FYM enriched with 2 kg each of the biopesticides at 1 kg/m<sup>2</sup> recorded maximum yield (56.66 t/ha), minimum nematode population (15 per 5 g root; 91 per 100g soil) and disease incidence of *Phytophthora capsici* (16.2%) and *Ralstonia solanacearum* (11.5%). The cost benefit ratio calculated for additional cost on biopesticides and additional returns gained by application of biopesticides was 1: 3.2.

### Management of late blight disease in kharif potato at Karnataka

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Late blight caused by *Phytophthora infestans* (Mont.) is one of the most dreaded diseases of potato worldwide and cause significant loss in production. The pathogen is highly variable and developed resistant to several fungicides. The outbreak of late blight was noticed in *kharif* potato crop 2006 onwards due to introduction of short duration exotic varieties which resulted in phenomenal reduction of *kharif* potato area from 48,000 to 18,000 ha in Hassan district of Karnataka. Hence, survey as well as management strategies was evolved for three years from 2012-14 to know the late blight appearance, severity and management of late blight. The late blight disease severity ranged from 4 to 40% and 0-100% during 2012-13 and 2013-14 respectively. Due to late blight, the expected yield loss was about 20-30%. Therefore, development of management practice for late blight in this area is must. Accordingly, an experiment was conducted to identify late blight resistant varieties/hybrids as well as fungicides schedule for management of late blight. Among the varieties tested, Kufri Girdhari recorded the minimum intensity of late blight (24.0%) followed by Kufri Shailja (30.0%) and Kufri Himalini (40.4%). However, highest yield was recorded in Kufri Surya (23.5 t/ha) followed by Kufri Himalini (20.9 t/ha). Among the schedule, prophylactic spray (just at the time of canopy closure) with mancozeb @ 0.2% followed by second spray 7-10 days after 1<sup>st</sup> spray with fenamidon + mancozeb @ 0.3%



and 3<sup>rd</sup> spray with mancozeb @ 0.2% 7-10 days after 2<sup>nd</sup> spray recorded lowest disease intensity (34.20%) and maximum yield (25.0 t/ha).

## **Management of *Phytophthora parasitica* causing Heart rot of Pineapple**

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Krishi Vigyan Kendra, Konehally, Tumkur

Pineapple (*Ananas comosa*) is an important commercial fruit crop grown in Soraba taluk of Shimoga district. The major constraints for pineapple growers in this region is heart rot caused by *Phytophthora parasitica* which results in serious economic damage when high rainfall occurs which is common in this area. In order to demonstrate an effective management practice front line demonstration was conducted in 5 farmers field in 2 ha area with farmer participatory approach with integrated heart rot management package involving selection disease free healthy suckers/crowns, pre plant dipping of planting material that is crown with 0.2% Metalaxyl Mz, soil application of *Trichoderma* enriched Neemcake @2q/acre and spraying with 0.1% Fosetyl al when disease noticed. It was observed from the demonstration plot that yield of pineapple was 495 q/ha compare to 340 q/ha in check plot further heart rot incidence was 32% in demonstration plot compare to 46% in check plot. In terms of economics demonstration plot recorded 3.20 BC ratio compare to 2.80 in check plot.

## **Field efficacy of new fungicide molecules against late blight of potato in Southern Karnataka.**

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Potato is an important vegetable crop of India and a staple food crop in many parts of the World. The crop suffers from many diseases. However, the occurrence of *Phytophthora infestans*, is a serious threat to the potato cultivation every year in South India. The field studies were carried out to evaluate new fungicides using the cultivar Kufri Jyothi. The disease intensity was recorded at 40, 50 and 60 days after sowing. The observations on disease development, per cent disease index, and yield per plot were recorded and the data was analyzed statistically. The results revealed that disease starts as brown or black water soaked lesions on leaves and stems and rapidly expands to cover the entire leaf area. In humid conditions it sporulates heavily causing visible whitish growth at the leading edge of lesions on abaxial surfaces of leaves. Among the different fungicides tested Azoxystrobin- 23 SC-1ml per litre is most effective with average per cent disease index of 13.63 and with the yield of 127.70 q/ha, followed by Fenamidon + Mancozeb -3 g per litre with per cent disease index of 24.50 and with the yield of 119.05 q/ha, and Cymaxanil +Mancozeb-3 g per litre with per cent disease incidence of 25.17 and with the yield of 115.48 q/ha. These chemicals were much superior over the standard check- Metalaxyl + Mancozeb-1.5 g per litre with average per cent disease index of 66.00 and with the yield of only 83.23 q/ha. The delay in application of fungicide by one day the effective fungicide also totally ineffective.



## **Phytophthora root rot of *Moringa oleifera* and its management**

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*Moringa oleifera* is commonly known as Moringa or drum stick, belongs to the family Moringaceae. The crop has attracted attention of scientists across the globe for its nutraceutical value. Root rot is prevalent in monoculture crop during rainy season or in irrigated areas affecting the main tuberized root causing yellowing of leaves and gradual drying up of the twigs. On uprooting and incubating the rotten part of tuberised root, fungal growth of *Phytophthora* sp. was noticed. The sporangial characters were matching with *P.nicotianae*. The culture isolated was able to produce root rot symptoms on healthy roots when inoculated in the laboratory. The pathogen was re-isolated to prove Koch's postulate. Among different fungicides evaluated in the laboratory bioassay using poison food technique, Copper hydroxide(3000ppm), Fenamidone+Mancozeb(3000 and 4000ppm), Fosetyl Al(3000 and 4000ppm) and Mancozeb(2000 to 4000ppm) completely inhibited the mycelial growth. Drenching of these chemicals was effective in complete recovery of plants from yellowing and drying symptoms due to root rot. Alternative to chemicals, few bioagents were tested against the pathogen. Among them, *B.licheniformis* S3b, *B. subtilis* S2b and *T. harzianum* exhibited 62.5 to 59.66% of inhibition. In areas where mono culturing of Moringa is being practiced it is suggested to amend the soil with these bioagents along with organic manure to maintain soil health.

## **Status and management of *Phytophthora* diseases in Coorg Mandarin**

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Coorg mandarin is a famous ecotype of mandarin grown in Kodagu region of South India for more than a century. Basically, Coorg mandarin is intercropped with Coffee and Pepper in this area. There has been a steady and constant decline over a period of time in the area under cultivation in this crop. Diseases incited by *Phytophthora* spp. are considered as one of the major causes of Coorg mandarin decline. It causes root rot, foot rot or collar rot or stump rot, gummosis, and brown fruit rot in the area. To ascertain the disease status, a survey was conducted in orchards growing Coorg mandarin in and around Chettalli, (Kodagu Dist.) In the orchard located in CHES, Chettalli disease incidence was highest (38.59%) the with disease severity of 13.05% whereas among coffee states growing Coorg mandarin as intercrop, Bellatti estate recorded highest disease incidence and severity (30.00% and 12.00 respectively) and the same were lowest in Laxmi estate (7.10% and 4.89%, respectively). On correlating the incidence of *Phytophthora* diseases with weather parameters and it was observed that with increase in humidity and rainfall there was a steep increase in *Phytophthora* infection. For the management of the disease, combination of different fungicides and biocontrol agents coupled with the application of Bordeaux paste were applied to the trees. Treatment of trees with Bordeaux paste and drenching of Al-fosetyl (0.2) was found most efficacious for management of *Phytophthora* root rot of mandarin.

## **Evaluation of chemicals and bioagents for managing *Phytophthora* root rot and root-knot nematode disease complex in capsicum under protected cultivation**

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Capsicum is an important spice cum vegetable crop of Karnataka state. Among the various diseases affecting capsicum, *Phytophthora* root rot (*Phytophthora capsici*) and root-knot nematode (*Meloidogyne incognita*) infestation are considered as the most destructive under protected cultivation. The study was carried out under polyhouse condition in Zonal Agricultural and Horticultural Research Station, UAHS, Shivamogga. An investigation was carried out to manage the disease using three fungicides (Copper oxychloride (0.1%), Metalaxyl MZ (0.1%), Mancozeb (0.2%)) in combination of nematicide (Carbofuran 3G) and three bioagents (*Trichoderma harzianum*, *Pacilomyces lilacinus*, *Pseudomonas fluoresces*). Management of disease will be most effective when chemicals are used with compatible bioagents compared to other methods of disease management. Hence, a chemical trial in combination with bioagents were used to manage the disease was carried out. The *Phytophthora* root rot disease was ranged from 60 to 70 per cent over untreated control. Among the tested chemicals, combination of Metalaxyl MZ + Carbofuran and *Trichoderma harzianum* were reduced the diseases incidence 82.00% with Root-knot index of 1.0 and Copper oxychloride was found to be the least effective.

## **Evaluation of chemicals for managing *Phytophthora capsici* and *Meloidogyne incognita* disease complex in Black pepper under poly house conditions**

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Black pepper (*Piper nigrum* L.) is an important spice crop of Kerala and Karnataka. Among the various diseases affecting black pepper, quick wilt (*Phytophthora capsici*) and slow wilt (*Meloidogyne incognita*) diseases are considered as the most destructive. Chemical control is the most effective and gives knock-down effect compare to other methods of disease management. Hence, a chemical trial to manage the disease was carried out in polyhouse condition using Bordeaux mixture (1%), Copper oxychloride (0.1%), Metalaxyl MZ (0.1%) and Akomin (0.2%) with combination of Carbofuran 3G (1g per pocket). The polybags of the vines were drenched with the treatments. The quick wilt disease control ranged from 71 to 82 per cent over untreated control. Among the tested chemicals, combination of Metalaxyl MZ + Carbofuran reduced the diseases incidence 82.00% with Root-knot index of 1.0 and Copper oxychloride was found to be the least effective.

**Session:9****Workshop on oomycete databases****FungiDB: An integrated functional genomics database for fungi and oomycetes.**

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FungiDB (<http://FungiDB.org>) is a free online database that enables data mining and analysis of the pan-fungal and oomycete genomic sequences and functional data. This resource was developed in partnership with the Eukaryotic Pathogen Bioinformatics Resource Center (<http://EuPathDB.org>). Using the same infrastructure and user interface as EuPathDB, FungiDB allows for sophisticated and integrated searches to be performed over an intuitive graphical system. Release 3.2 of FungiDB contains sequence and annotation for 20 Oomycete genomes including members of *Albugo* (2), *Aphanomyces* (2), *Hyaloperonospora* (1), *Phytophthora* (6), *Pythium* (7), and *Saprolegnia* (2). In addition to the genomic sequence data and annotation, FungiDB includes transcriptomic data based on RNA sequence and microarray experiments and all expressed sequence tag data from GenBank. All genomes in FungiDB are run through a standard analysis pipeline that generates additional data such as signal peptide and transmembrane domain predictions, GO term and EC number associations and orthology profiles. The graphical user interface in FungiDB allows users to conduct *in silico* experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in *Phytophthora sojae* that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. FungiDB is supported in part by the Burroughs Wellcome Fund, the Alfred P. Sloan Foundation, USDA, NIFA and NIH HHSN272201400030C.

## **EuMicrobedb: A genome database and annotation interface system for Oomycetes pathogens.**

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We describe EuMicrobedb – A light weight genome database and annotation system for oomycetes organisms. The earlier version of Eumicrobedb was VMD (VBI Microbial database: [www.vmd.vbi.vt.edu](http://www.vmd.vbi.vt.edu) ). It was based on Genome Unified Schema (GUS) and was heavily dependent on third party softwares including commercial packages such as Oracle. In order to overcome the drawback of the large size of schema and proprietary nature of oracle software, we rebuilt a much light weight version of the schema using Mysql. We have removed dependencies on bioperl object layers for making the system more portable. Eumicrobedb now runs on a powerful Light weight annotation engine that we have developed called “Genome Annotator Lite”. Currently this database has 20 oomycetes genomes and 10 EST datasets. The genomes have been compared with each other using LastZ and plotted on the genome browser. Additional feature tracks such as ESTs, tRNAs, repeat tracks are also included wherever available. We have curated conserved genes across 10 oomycetes species and generated conserved clusters. The main annotation page (browserDetail Page) is the central resource for each gene and the most downstream component of the database. All pages eventually open to this page, where descriptions about a gene are available in great detail. Query-able comparative genomics and conserved orthologous genes among oomycetes is one of the new and key features of this database. The browser has been upgraded to enable user upload of GFF (genome feature file) files for quick genome annotation comparison. The database system is available at [www.eumicrobedb.org](http://www.eumicrobedb.org) .





## Session: 10

### Interactive meeting between scientists and stakeholders

#### **DuPont™ Zorvec™ (“DPX-QGU42”, “oxathiapiprolin”): The first member of a novel class of oomycete fungicides**

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DuPont™Zorvec™ is the global branded name for oxathiapiprolin (approved ISO common name), a novel fungicide recently discovered by DuPont and the first member of a new class of piperidinyl- thiazole-isoxazoline fungicides. It acts at a unique site of action in Oomycete pathogens. High intrinsic efficacy, an effect on multiple stages of pathogen development and systemic movement within the host plant allow oxathiapiprolin to provide robust and reliable disease control even under the most severe conditions. Product development is focused on crops where Oomycete pathogens limit agricultural productivity and profitability including potatoes, grapes, cucurbits and other vegetable crops. At use rates 5-100 times lower than current commercial fungicides, oxathiapiprolin is highly effective for the control of important Oomycete pathogens including; *Phytophthora infestans*, *Phytophthora capsici*, *Plasmopara viticola* and *Pseudoperonospora cubensis*. Its new mode of action makes oxathiapiprolin a valuable option for fungicide resistance management strategies, while safety to key beneficial organisms confer a strong fit within integrated pest management programs. A remarkably favorable toxicological and environmental profile, combined with low use rates, provides large margins of safety for consumers, agricultural workers and the environment.

#### **Integrated management of *Phytophthora* diseases of cocoa**

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Agricultural production of several crops is significantly reduced in wet tropics due to large number of different diseases caused by *Phytophthora*– ‘the plant destroyer’. Among the *Phytophthora* species infecting cocoa in India, *P. palmivora* is the most important pathogen and infects all parts of the plant causing black pod disease (BPD), stem canker(SC), foliar infection, chupon blight, twig dieback, root rot and seedling blight/dieback in nurseries.

To develop economically feasible and viable *Phytophthora* disease management strategies, it is



very much essential to have a thorough understanding of some of the special characteristics of this pathogen, and certain factors contributing to the highly pathogenic nature of this pathogen. It can survive within host tissue as well as outside host tissue for long periods. Soil acts as a reservoir of *Phytophthora* in disease endemic areas. Congenial climatic conditions during rainy season in the tropical low lands and heavy mist and dew producing free water throughout night in tropical high lands are very much favourable for the pathogen to produce abundant infective propagules. Some of the characteristics of cocoa also make a favourable condition for the establishment of *Phytophthora* and for disease severity. A single species of *Phytophthora* can infect different parts of cocoa and several species can infect a single host plant. Several insects act as vectors for the fast spread of *Phytophthora* diseases. In India, numerous slugs (*Deroceras* spp.) are found voraciously feeding on the *Phytophthora* growth on cocoa pod surface. Infective propagules are found in the feces of these slugs. The fecal matter is found very infective. Such observations are indirect evidences for the major role of *Deroceras* spp. as dispersal vector of *Phytophthora*. Non-adoption or improper adoption of some of the agronomic practices acts as pre-disposing factors for disease spread and its severity.

A thorough knowledge of the various factors that make *Phytophthora* species such an efficient pathogen of cocoa is very much imperative in developing proper integrated management strategies. It is not possible to control *Phytophthora* disease with fungicide treatment alone. Therefore, a combination of different practices such as disease-free planting material, plant and field hygiene, orchard management including nutrient management and irrigation and fungicide and/or biocontrol agent application in an integrated manner have to be adopted to get any success in *Phytophthora* disease management. BPD could be effectively controlled when fungicide application is integrated with cultural practices. *Trichoderma* treatment using *Trichoderma* coir pith cake was found to be effective in the management of SC. It is expected that *Trichoderma* treatment will be more effective if continued year after year. Investigations on various aspects of cocoa black pod and stem canker diseases caused by *P. palmivora* in India have helped to evolve a combined integrated disease management strategy with more emphasis on cultural practices for BPD and SC diseases. Integrated disease management strategy developed in India for raising cocoa seedlings during rainy season is very effective. It is important to fully integrate *Phytophthora* disease management practices to overall farm management through a holistic approach.

### **Managing fungicide resistance in *Phytophthora* caused plant diseases**

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*Phytophthora* diseases cause devastating losses to the plants around the world and their effective and sustainable management continues to be a challenge for plant pathologists. Due



to lack of stable host resistance, fungicides play a significant role in managing this destructive pathogen and realizing optimum crop yields. The process of fungicide discovery for managing *Phytophthora* diseases has undergone a significant change over the years. After the early era of broad spectrum multisite fungicides like dithiocarbamates, copper fungicides, phthalimides, chloronitriles and others, phenylamides, particularly acylalanines provided a much needed relief in the management of *Phytophthora* diseases. Over the past two decades, several novel action fungicides of different chemical classes have been developed for use against *Phytophthora* and other oomycetes. Prominent among these include strobilurins, oxazolidinediones (famoxadone), imidazoles (fenamidone), benzamides (fluopicolide, zoxamide), valinamides (iprovalicarb, benthiovalicarb), mandelamides (mandipropamid), cyanoimidazoles (cyazofamid), and thiocarbamates (ethaboxam) representing different chemistries and modes of action.

Rapid development of resistance to phenylamides, notably to metalaxyl, in *Phytophthora* and related oomycetes is well documented. Experience with the Q<sub>0</sub>I fungicides worldwide indicates that these fungicides are also highly prone to resistance build up in target pathogens. To reduce the resistance risk, these need to be used in pre-packed mixtures. Synergic fungicide mixtures containing a phenylamide component and a multi-site contact fungicide are still an effective strategy for the control of late blight in potato and tomato. New compounds with novel modes of action are good options to manage phenylamide resistance. It is critical to use an effective disease management program to delay the build-up of resistant *Phytophthora* sub-populations. The main principle for managing fungicide resistance is aimed at delaying its development. A management strategy, therefore, should be implemented before resistance becomes a problem. The major approaches for the management of fungicide resistance in practice include avoiding sole use of at risk fungicide, avoiding post-symptom curative applications, use of cultural practices, reduction in number of applications using them early in the season, application at proper dose rate, integration with cultural and other control practices, avoiding soil application etc. The use of new generation fungicides needs to be regulated and FRAC guidelines adopted so as to maintain their efficacy levels.

## ***Phytophthora* diseases of apple and their management in India**

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Apple (*Malus domestica* Borkh.) is a major fruit crop grown in Himalayan region of Asian and other temperate zones of the world. In India, apple is being grown over an area of 311.5 thousand ha, with an annual production of 1915.4 thousand MT with average productivity 6.1 t/ ha, which is quite low. Amongst different diseases of apple, collar rot, crown rot, root rot and fruit rot caused by *Phytophthora cactorum* (Leb. & Cohn) Schroeter and its other species is most severe both under nursery as well as orchard conditions, and thus lead to huge economic losses. Incidence of collar rot in apple under nursery and orchard conditions of Himachal Pradesh varied between 2.5-24.5 and 0.2-77.5 percent, respectively. Tree mortality of 5-35

per cent has been reported in different parts of Kashmir. It is very serious in some parts of Uttarakhand and Arunachal Pradesh due to favourable soil conditions. Out of different species of *Phytophthora* identified to cause collar/ crown/ root rot and fruit rot in apple, *Phytophthora cactorum* was identified the most common pathogen to cause collar rot with an average frequency of occurrence ranging between 78-89 per cent. Other species of *Phytophthora* viz., *citricola*, *cambivora*, *citrophthora*, *syringae* and *litorale*-a new record were also identified. Fruit rot was mainly observed to be caused by *P. cactorum* and *P. syringae*. Presence of *Pythium ultimum* further aggravates the disease incidence and is the next common pathogen with its frequency ranging between 12-22 percent. Geographic distribution map of *Phytophthora* in apple orchards of Himachal Pradesh was developed by adopting modified standard SADAMCAP (Soil –Air-Dried and Moistened Chilled and Plated) method. In disease development studies in relation to soil factors, it was concluded that the occurrence of mean soil temperature ranging between 18.3-22.7°C up to 10 cm depth during the month of April and August –September accompanied with the prevalence of soil moisture ranging between 68.3-82.3% per cent up to 10 cm depth was highly conducive for disease initiation and subsequent spread. Disease development was positively correlated with rainfall, soil moisture and minimum soil temperature and negatively correlated with maximum soil temperature. Out of 27 pollinizer cultivars, *Malus floribunda* showed maximum tolerance with minimum mortality of 11.1 and 9.4 under pot and nursery conditions, respectively. It was followed by Stark spur Golden, Bray Burn, Star Crimson and Winter Delicious in order. Different individually highly effective management inputs against the target disease in apple in the earlier studies (2008-11) were further evaluated in different combinations under pot, nursery (sick plot) and orchard conditions during 2012-14 to develop an integrated management strategy. Under nursery conditions, soil application of biocontrol agents (*Trichoderma harzianum* 5 (TH5)/ *T. hamatum* 2 (THM2)/ *T. viride* 5(TV5)/ *T. harzianum* 15 (TH15) @150g wheat bran culture + 10 g talc formulation + *Bacillus* sp. 11 (BS11) /*Bacillus*-4 (BS4)/ *Enterobacter aerogenes* -2: EA2 (150g coconut coir +10 g talc formulation) combined with mustard cake (200g)/ bio-fumigation with mustard plants (2kg)/ *Murraya koenigii*/ *Eucalyptus* leaf amendments (200g) and fungicide Curzate (0.3%) or Cabrio Top ( 0.2%) or Ridomil Gold (0.3%) per m<sup>2</sup> plot under nursery conditions were effective and provided almost complete control (>98%) of disease when applied prior to planting of apple seedlings/ susceptible rootstock (MM106). Soil solarization was adjusted as one of the most important management input under nursery conditions. Under orchard conditions, combined applications of biocontrol agents viz., TH5/TH15 (200g bran culture + 50 g talc formulation), *Bacillus* sp.11/EA2 (200g coconut coir culture + 50 g talc formulation) added in the first week of April and last week of August, bio-fumigation with mustard plants @ 2kg (first week of March) and Curzate (cymoxanil 8% + mancozeb 64%) @ 0.3% (April and August) was quite effective and increased the shoot growth to 37.4 cm against 6.8 cm in untreated plants. Three sprays with fosetyl-Al (Aliette) at pea stage, walnut stage and fruit development stage were also effective and recommended in controlling both collar and fruit rots. In an another trial, combined application of red soil (10 cm layer) along with TH5, BS11and Curzate was also effective in increasing the shoot length 41.9 cm as against



6.1cm in control untreated plants with lesion healing up to an extent of 36.1 per cent. Addition of cow urine decoction (prepared from leaves of *M. koeningi*, Eucalyptus and seeds of *Melia azedarach* in cow urine) thrice @7.5% (10lts/tree) during March, June and August accompanied with approach grafting (AG) and two applications of Curzate (0.3%) during April and August was also quite effective.

### ***Phytophthora* diseases of citrus in India**

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*Phytophthora* spp. cause serious diseases like root rot, crown rot, foot rot, gummosis and brown rot of fruits in citrus inflicting decline and yield losses in India. A total of 198 isolates belonging to 8 different *Phytophthora* spp (141 isolates of *P. nicotianae*, 36 isolates of *P. palmivora*, 6 isolates each of *P. citrophthora* and *P. insolita*, 4 isolates of *P. boehmeriae*, 2 isolates of *P. tropicalis*, 2 isolates of *P. macrochlamydospora* and 1 isolate of *P. lacustris*) were isolated from rhizosphere soil and water, citrus root, leaf, bark and fruit samples collected from 12 major citrus growing states of India. Morphological characterization coupled with PCR-RFLP based system of the ITS region of the genomic rDNA was used as a tool to identify all the seven *Phytophthora* spp. Sequencing and phylogenetic analyses of the ITS region, beta tubulin gene, translation Elongation factor 1 alpha and the region containing the mitochondrial cytochrome c oxidase subunit 1 and 2 gene fragments also confirmed the identity of these species. Phylogenetic analysis revealed that ITS and other gene sequences of *P. nicotianae* and *P. palmivora* isolates derived from citrus orchards of India and other parts of the world were highly conserved and variation was not geographically confined. Intra-species variation was observed more in *P. nicotianae* isolates than in *P. palmivora* isolates. Species-specific primer pairs NIC1/ NIC2 and Pal1s / Pal2a were successfully tested for specific detection of *P. nicotianae* and *P. palmivora* isolates, respectively. These two *Phytophthora* species were also detected in citrus roots and rhizosphere soils using nested PCR and PCR RFLP techniques. The findings relative to morphological and molecular characterization of *Phytophthora* spp. obtained from Indian citrus plantations and its implications for pathogen identification and disease detection would be discussed.

### ***Phytophthora* diseases of cassava and taro**

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Tropical root and tuber crops serve as a major staple for one fifth of the world's population. They are a rich source of carbohydrate and play a vital role in reduction of hunger, enhancing food security and promise as alternative crops of the future. Among them cassava is the most important subsistence food and industrial crop of the developing countries. In India, cassava

[*Manihot esculenta* (L.)] is grown as a subsidiary food crop in Kerala while it is a major industrial crop in Tamil Nadu (TN) and Andhra Pradesh (AP). More than 60% of the area under cassava in TN are around Salem belt comprising Salem, Erode, Namakkal and Dharmapuri districts popularly known as the “Land of Sago” even in International forums. The major constraint that restricts the cassava production in these areas is the occurrence of tuber rot disease caused by a soil borne pathogen, *Phytophthora palmivora*. Uncontrolled irrigation, poor drainage systems and high rainfall during September – November is the most conducive environment for the pathogen to multiply in the soil which infects & rots the tubers. A yield loss up to the tune of 70% has been reported in Tamil Nadu and Kerala. The disease is characterized by the appearance of a dark colored round to irregular shaped water soaked lesions on mature tubers in the field. On advancement of infection, the lesions enlarge causing internal browning, oozing of internal fluids and shriveling of the tubers. Subsequently, the infected tubers emit a characteristic foul smell and rot within 5-7 days depending on the soil conditions making the tubers useless. *P. palmivora* isolates collected from Tamil Nadu and Kerala were characterized through morphological and molecular (RAPD and RFLP) methods. The results of these studies revealed the presence of high levels of genetic diversity among the isolates. To facilitate early and accurate detection of *P. palmivora*, a PCR assay was developed by designing specific PCR primers which was found to be highly successful. Deep ploughing, controlled irrigation, ridge planting and application of neem cake and *T. harzianum* could manage the disease successfully.

Taro [*Colocasia esculenta* (L.) Schott], is also a major tuber crop with wide distribution in the tropics and serves as a major staple in the diets of people around the globe and it is the fourteenth most consumed vegetable worldwide. The taro plant is a rich source of carbohydrates, proteins, minerals and vitamins and has medicinal properties to reduce tuberculosis, ulcers, pulmonary congestion and fungal infection. Leaf blight and corm rot caused by *Phytophthora colocasiae*, is the most destructive disease of taro. The first symptoms of *Phytophthora* blight appear as small, dark brown flecks or light brown spots on the upper leaf surface, which enlarge rapidly, and destroy the leaf within 3-5 days, depending on the weather conditions. Epidemics are common when night and day temperatures range between 20-22 and 25-28°C, respectively, with a relative humidity of 65% during the day and 100% at night and accompanied by overcast, rainy weather. *Phytophthora colocasiae* isolates collected from taro growing regions throughout India were characterized by morphological methods and ITS rDNA sequencing. RAPD and AFLP markers revealed the presence of high levels of diversity among the isolates irrespective of their origin or phenotypic characters which was also evident in isolates even from fine spatial scale (isolates from multiple blight lesions on a single taro leaf) assessed using RAPD marker. For early and accurate detection of *P. colocasiae*, specific primers were designed (using RAS-like, TRP1, GPA1 and ASF-like regions) and Real-time quantification (qPCR) of pathogen is being standardized. Disease free planting material and crop rotation are the primary recommendations for managing the disease. The cvs. ‘Jankhri’ and ‘Muktakeshi’ were reported as highly tolerant to blight which could be used in breeding programmes. The biocontrol agents *Pseudomonas* and *Trichoderma* spp were effective in reducing the intensity. Recently, the application of vermiwash and vermicompost was found to reduce the disease intensity in the field conditions.





Regarding chemical fungicides, copper and metalaxyl fungicides have proved to be very effective in controlling leaf blight. Eventhough detailed studies have been conducted to study the status of these diseases, pathogens, survival and management, there is a need to develop resistant varieties and decision support system.

### ***Phytophthora* diseases in spice crops**

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Indian Spices are preferred in the world market due to its intrinsic qualities. The major crops are black pepper, cardamom, chilies, cinnamon, clove, nutmeg , mace and vanilla. One of the major production constraints in these crops is the diseases caused by *Phytophthora*. The onset and spread of the disease is severe during the wet south-west monsoon period commencing from June to the end of September. Most of these crops are cultivated as intercrops in cropping system involving palms, coffee and tea. The microclimate favour the incidence and spread of these diseases. The diseases can be managed only with the adoption of integrated management strategies rather than adopting individual disease control measures.

In perennial cropping system when the congenial conditions occur only at a certain months in a year partly infected plant serves as source and focus of secondary spread. Removal of infected plants reduces the initial inoculum for the next season. Being a wet weather pathogen and exacting in its weather requirements, monsoon period provides such a congenial condition and a fixed prophylactic chemical spray is preferred to forewarning. Most often the pathogen is introduced inadvertently from diseased nurseries. Hence, production of disease free planting material preferably in soil free rooting medium fortified with bioagents plays a crucial role in disease management. There are proven biocontrol agents and growth promoting rhizobacteria to manage *Phytophthora* infection in spice crops. Delivering the beneficial organism is another point to be emphasized in disease management. The schedule followed in managing *Phytophthora* diseases in spice crops are discussed in the paper.

### ***Phytophthora* diseases of vegetable crops**

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India is the second largest producer of vegetables in world with an annual production of 87.53 million tonnes from 5.86 million hectares, accounting 14.4% to the world production. Diseases caused by *Phytophthora* spp are emerging as a major production constraint for sustainable vegetable production in India. Since 2008, severe outbreaks of *Phytophthora* diseases such as late blight on tomato (*P. infestans*), fruit rot on brinjal and cucurbits (*P. parasitica*), foliar blights and wilts in chili (*P. boehmeriae* and *P. capsici*) were noticed. *P. infestans* isolates causing late blight of tomato, collected from south-west India, were assessed for metalaxyl sensitivity,



mating type, mitochondrial DNA (mtDNA) haplotype, DNA fingerprinting patterns based on simple sequence repeats (SSR) and RG 57 probe and aggressiveness on tomato. All isolates were metalaxyl resistant, A2 mating type, mtDNA haplotype Ia and had identical SSR and RG57 fingerprints and highly aggressive on tomato. The phenotypic and genotypic characters of fungal isolates were found to be similar to that of 13\_A2 genotype of *P. infestans* population reported in Europe. The 13\_A2 MLG is unrelated to *P. infestans* populations prevalent in other Asian countries suggesting migration as a more likely cause of the emergence of this pathogen in India. India imported potatoes from U.K and from Netherlands during 2006 and 2007 where 13\_A2 genotype was found since 2005 and this genotype might have migrated to India along with potatoes. *Phytophthora* isolates, recovered from blight affected leaf tissues of hot pepper from different localities in Karnataka and Tamil Nadu states between 2011 and 2012, were identified majority of the isolates as *P.boehmeriae* and few isolates as *P.capsici* based on morphology, a similarity search of ITS sequences at GenBank, PCR-RFLP patterns and species-specific PCR using PC1/PC2 and PB1/PB2 primer pairs. All isolates of *P. boehmeriae* were metalaxyl sensitive while *P.capsici* isolates were intermediate in sensitivity. *P.boehmeriae* isolates were highly aggressive and produced significantly larger lesion than those of *P. capsici* isolates. Phenotypic and genotypic characterization of *Phytophthora* isolates associated with brinjal and cucurbits is in progress.

Thus, the migration of 13\_A2 genotype *P. infestans* was the cause of outbreaks of destructive late blight epidemics in India and stresses the importance of bio-security in agricultural trade. The emergence of *P.boehmeriae* was responsible for severe leaf blight epidemics on hot pepper in South India although it is not serious pathogen on any crop in any part of the world. These invasive and emerging *Phytophthora* species have epidemiological and management implications for the production of vegetable crops in India.

### ***Phytophthora* diseases of palms**

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Coconut and arecanut are the two major agriculturally important palms cultivated in humid tropics of India. *Phytophthora* “the plant destroyer” causes severe devastating diseases in both these palms. Among the diseases affecting coconut palm, bud rot caused by *Phytophthora palmivora* is a serious fatal disease causing huge loss to farmers. Though the disease is sporadic in nature, outbreaks of epidemic are also common during favourable climatic conditions especially in years of prolonged rainy season. The intensity of the disease is increasing year after year due to build up of inoculums in the endemic areas of India causing severe economic loss. Though the *P. palmivora* is the major pathogen causing bud rot, association of other *Phytophthora* species has been reported in rare occasions. Integrated management practices for bud rot have been developed. A *Trichoderma harzianum* isolate effective against *P. palmivora* has been formulated with coconut coir pith and found very useful for management of bud rot. Apart from bud rot, *P. palmivora* also infects immature coconut and cause immature nut fall. In case of arecanut, fruit rot, bud rot and crown rot are the severe diseases caused by *Phytophthora*. Fruit



rot of arecanut caused by *Phytophthora meadii* is a devastating disease which leads to severe shedding and rotting of nuts. The disease may cause fruit drop of 50 to 100 per cent in individual palms if timely and proper control measures are not adopted. Fruit rot disease occurs in all the arecanut growing regions receiving heavy rainfall during southwest monsoon period. Bud rot or crown rot is another manifestation of fruit rot and this may occur independently or following severe fruit rot. The fruit rot may cause loss of nuts during the particular season; however bud rot and crown rot are fatal and kill the tree. Over the years various chemicals were screened for the management of the *Phytophthora* diseases in coconut as well as arecanut. Fungicidal spray could control the disease only to a limited extent as the heavy rains resulted in washing off of the fungicides from plant surface. An integrated disease management involving proper cultural practices, application of biocontrol agents, fungicidal sprays and host-plant resistance need to be employed for effective management. It is easier said than practice the effective control measures for *Phytophthora* disease in the perennial trees like coconut and arecanut grown in high rainfall areas. Especially the small and marginal farmers are facing difficulty in adopting timely control measures due to shortage of skilled labours. Suitable easy to operate labour saving mechanical devices for application of fungicides are the need of the hour. Various issues and strategies for the management of *Phytophthora* diseases in coconut and arecanut are discussed in the paper.

### ***Phytophthora* diseases in ornamental crops in India**

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Commercial floriculture is a fast growing industry in India. Cultivation of crops like rose, carnation, gerbera, gladiolus, tuberose, crossandra, marigold and jasmine is undertaken both by small farmers, who usually meet the local requirements and also by others on commercial scale mainly to meet exports. Diseases, insect pests and weeds constitute a major constraint in raising ornamental crops of good quality. Among the various fungal pathogens of flower crops, *Phytophthora* species cause a serious crop loss. Important *Phytophthora* diseases in ornamentals include foot rot in carnation by *P. palmivora*, *P. cactorum* or *P. citricola*; foot rot by *P. cryptogea* in gerbera; black rot or leaf blight by *P. nicotianae* and *P. citrophthora* in anthurium; foot and root rot by *P. nicotianae* in crossandra and black or heart rot by *P. palmivora* and *P. parasitica* in orchids. These diseases can be managed using conventional contact fungicides like Bordeaux mixture, copper oxy chloride or mancozeb along with judicious use of metalaxyl. Systemic fungicides viz. iprodione, fosetyl Al, metalaxyl, fenamidone + mancozeb, famoxadone + cymoxanil etc. are also used extensively to manage these diseases. Extensive use of systemic fungicides poses the problem of fungicide resistance. In India use of biological control agents is gaining momentum. For managing the soil inoculum of *Phytophthora* application of *Trichoderma* enriched compost along with seed or planting material treatment with bioagents has resulted in very good management of diseases due to *Phytophthora* in many crops that need to be extended to ornamental crops. The disease problems caused by *Phytophthora* species in important ornamental crops and various options available to combat them are discussed in this presentation.

## ***Phytophthora* diseases of rubber**

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Abnormal leaf fall (ALF), shoot rot, patch canker and black stripe are the diseases caused by *Phytophthora* spp. on rubber trees. On young rubber and in nursery plants, shoot rot occurs leading to extensive die back and growth retardation. In mature rubber trees, extensive defoliation due to ALF disease leads to considerable loss of crop. ALF is one of the most destructive and economically important diseases of rubber, occurring during the southwest monsoon period. Crop loss studies revealed 7-31 per cent loss due to ALF disease depending on the clone. It was reported that a continuous spell of 250 to 350 mm rainfall for a period of 7 to 10 days without intermittent hot sunshine, with minimum and maximum temperatures within the range of 22-25°C and 26-30°C respectively and relative humidity above 90 per cent are the most congenial conditions for the outbreak of ALF disease.

An efficient cultural technique adopted for management of *Phytophthora* disease on rubber tree is crown budding, wherein the susceptible canopy of the high yielding clone is modified using disease tolerant crown for better leaf retention. Prophylactic spraying with copper fungicides is recommended to contain the disease. As the maximum potency of copper fungicides lasts only for 4 to 6 weeks, spraying needs to be done as close to monsoon as possible. Spraying with 1 per cent Bordeaux mixture using high volume sprayers or oil-based copper oxychloride (COC) dispersed in agricultural spray oil (1:5 proportion) using low volume sprayers is effective. For micron spraying, 30-40 L/ha. of COC-oil mixture is required. Single man or four men carrying or tractor mounted micron sprayers are recommended for low volume spraying. A mini-tractor mounted mist blower has also been recently recommended for delivering the spray fluid to a height of up to 100 feet. Research on protection of rubber trees against *Phytophthora* diseases has been successful mainly due to the use of effective chemical fungicides coupled with efficient spray technology to deliver the fungicides on tall rubber trees.

### **Zorvec™ an effective fungicide for the management of downy mildew in grapes in South Asia**

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Grape (*Vitis* sp.) belongs to Family Vitaceae and is the most important commercial fruit crop in India. This crop occupies fifth position amongst fruit crops in India with a production of 1.21 million tonnes (around 2% of world's production). It is a temperate crop which has got adapted to sub-tropical climate of peninsular India. Downy mildew is the most devastating disease of grapes in the tropical areas of South Asia. The disease not only impacts leaves, but flowers



and berries also. If the disease is not effectively managed, this may lead to 100% crop losses. The new fungicide **Zorvec™** was tested against this disease for the management of downy mildew in grapevine. Amongst the different treatments, **Zorvec™** was found most effective in disease control and helped in enhancing the yield. Maximum yield was recorded from the **Zorvec™** treatment. Further, there were no phytotoxic effects of **Zorvec™** observed on grape cultivar. In the event of high disease pressure, the new product can effectively be employed for the management of downy mildew in grapevine ecosystem and this can prove to be an effective tool in future to tackle the resistance development issues.

### **Zorvec™ a new age fungicide for the management of late blight in Potato in South Asia**

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The need for assured food security to the ever increasing population and shrinking availability of arable land has necessitated temporal and spatial intensification of cropping. Short duration cash crop of potato, which is highly amenable to adjustment, fits in well in various cropping systems. The potato (*Solanum tuberosum* L.) was introduced to southern Asia in the late sixteenth century, by Portuguese mariners. In India potato is presently grown under diversified agro-climatic conditions. India ranks fourth in potato area (1.48 million ha) and third in production (28.47 million tonnes) in the world with an average yield of 183q/ha. Potato is infested by a plethora of insect pests and diseases and amongst them Late blight of potato caused by *Phytophthora infestans* is one of the most devastating disease of potato and can cause yield losses upto 85% if crop (susceptible cultivar) remains unprotected. Disease appears every year in epiphytotic forms in hills as well as in plains. Under highly congenial conditions late blight appears in epiphytotic form and kills the entire foliage within a few days. Yield losses due to late blight varies from year to year and ranges from 19-65 % in eastern hills, 11-74 % in North western hills, 10-75 % in Eastern plains, 20-40 % in north western plains and 31-39 % in southern hills. The new fungicide **Zorvec™** was tested against this disease across the south Asia and the results across different geographies indicated superior performance of this product at very low use rate. The results not only indicated superior disease protection for longer duration but protected the loss of yield when compared to any of the commercial standards.







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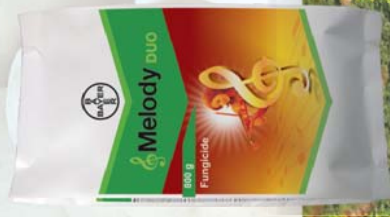


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Number of leaves	5-7 functional leaves
Height of the graft	30-45 cm
Height of the graft Joint	15 -20 cm from collar region.
Growth	Healthy and vertical growing
Graft joint	Perfect without any girdling or constriction.
Nature of Polythene bag	Intact and not torn
Side sprout	Free from side sprout from the root stock

## Quality standards - Cocoa hybrid seedlings

Characters	Standards
Age of the seedling	5-6 months
Number of leaves	5-6 pairs
Height of the graft	45-50 cm
Growth	Vigorous seedlings growing straight at the middle of the poly-bag.
Jorquetting	No jorquetting



Directorate of Cashewnut and Cocoa Development (DCCD) , Kochi is the national agency approved by Government of India for accreditation of cashew/cocoa nurseries. Application and other guidelines for accreditation is available in DCCD website [www.dccd.gov.in](http://www.dccd.gov.in).



## काजू और कोको विकास निदेशालय DIRECTORATE OF CASHEWNUIT AND COCOA DEVELOPMENT

कृषि मंत्रालय, Ministry of Agriculture

कृषि एवं सहकारिता विभाग, Department of Agriculture and Cooperation

केरा भवन, कोच्ची - 682011, Kera Bhavan, Kochi-682011

Web: <http://dccd.gov.in>, E-mail : [dccd@nic.in](mailto:dccd@nic.in) Phone: 0484 -2377151 Fax: 0484- 2377239



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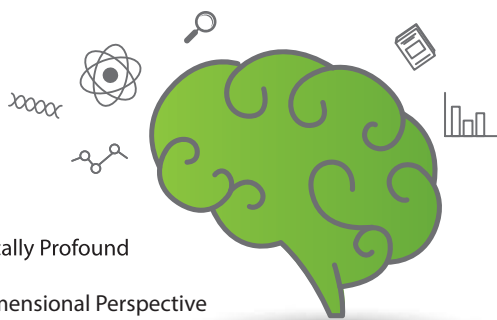


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# Government of Karnataka Department of Horticulture



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