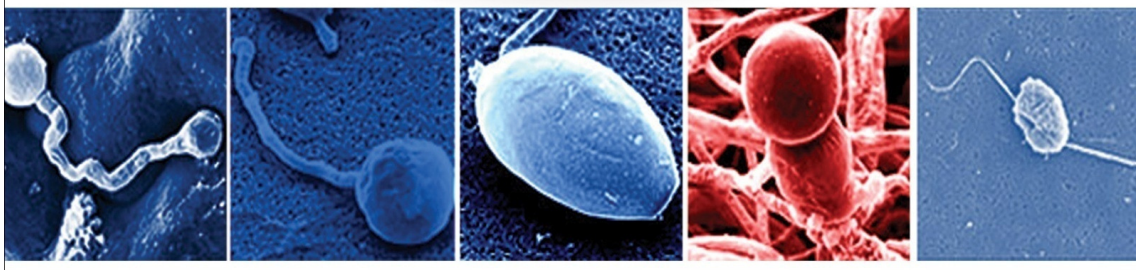
Proceedings of Final Review Meeting of

Outreach Project on *Phytophthora*, *Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops

on 4th March 2012



भारतीय मसाला फसल अनुसंधान संस्थान
Indian Institute of Spices Research

CONTENTS

PROCEEDINGS OF THE FINAL REVIEW MEETING OF PHYTOFURA

OUTREACH PROJECT ON *PHYTOPHTHORA*, *FUSARIUM* AND *RALSTONIA* DISEASES OF HORTICULTURAL AND FIELD CROPS

held at
Indian Institute of Horticultural Research, Bengaluru on 3-4th March 2011

The final review meeting of the outreach project on *Pytophthora, Fusarium* and R*alstonia* diseases of horticultural and field crops (PHYTOFURA) along with that of ALCOCERA was held at Indian Institute of Horticultural Research, Bengaluru on 3-4th March 2011 under the chairman ship of Dr H.P. Singh**,** Deputy Director General (Horticulture, ICAR , New Delhi. Dr. A. M. Mukhopadhyay, Former Vice Chancellor, Assam Agricultural University, Jorhat and Dr. J. Kumar, Dean & Registrar, GB Pant University of Agriculture and Technology, Pantnagar attended the meeting as experts for review. Many scientists from all the involving centers participated in the review meeting.

Dr Amrik Singh Sidhu, Director, IIHR welcomed the gathering and hoped that the project made significant contributions to our present knowledge on these organisms.

Many technical bulletins were released. They are

1. Final Annual Report of ALCOCERA (2009-2012)
2. Salient Achievements of PHYTOFURA
3. DNA Bar-coding of economically important fungal species of field and horticultural crops
4. Integrated management of early and late blight of potato and tomato
5. Bordeaux mixture

Dr. P. Chowdappa, Principal Scientist, IIHR and coordinator presented the consolidated report and progress of ALCOCERA. Dr. M. Anandaraj, Director, IISR and coordinator presented the consolidated report and progress of PHYTOFURA. The genesis and the onward progress of the project and the guidance and encouragement given by the honorable DDG Dr H.P. Singh for smooth running of the project was highlighted by both the coordinators.

Dr H.P. Singh in his remarks lauded the efforts of both the coordinators for the significant achievements of both the projects and said the stupendous amount of information generated in the project helped us to have much better understanding of the host, pathogen and environment interactions involved for developing better management strategies for safer and green environment. He complemented the efforts of both the projects in generating whole genome sequences of *Phytophthora , Alternaria, Colletotrichum* and *Cercospora* and said now in the 12th plan we must focus on developing facilities and expertise for annotation of these voluminous sequence data generated. He also advised that the final report of the project must clearly categories the results obtained into - Achievements in terms of targets fixed for each activity, Questions-Answered, Process/ Good Agricultural P practices / Product/ Genetic stocks/ Technology/ Software/ Expert systems/ Databases Developed their practical Utility and the revenue the project is expected to generate in future years. He also suggested that future lines of work must be clearly defined and expressed strong conviction that this project will continue in the 12 plan also.

Dr M. Krishna Reddy, HOD, Plant Pathology, IIHR gave vote of thanks and informed the house that whole genome of Ralstonia was also sequenced at IIHR, Bengaluru.

The individual review of ALCOCERA and PHYTOFURA project was done on 3rd and 4th March 2012 respectively. Dr. A. M. Mukhopadhyay, Former Vice Chancellor, Assam Agricultural University, Jorhat Chaired and reviewed the progress and Dr. J. Kumar, Dean & Registrar, GB Pant University of Agriculture and Technology, Pantnagar was the co-chairman.

REVIEW OF PHYTOFURA ON 4th MARCH 2012

Chairman: Dr. A. M. Mukhopadhyay, Former Vice Chancellor, Assam Agricultural University, Jorhat

Co-chairman: Dr. J. Kumar, Dean & Registrar, GB Pant University of Agriculture and Technology, Pantnagar

The organisms, crops and the corresponding institutes involved are given below.

Sub-project I: *Phytophthora*

Crop	***Institutes***
Black pepper	IISR, Calicut
Potato	CPRI, Shimla
Citrus	NRC, Citrus, ICAR RC NEH, Umiam
Coconut and cocoa	CPCRI, Kasaragod
Colocasia	CTCRI, Trivandrum
Apple	YSPUHF, RC Kullu
Biological Control	NBAII, Bengalure
Conservation & characterization	NBAIM, Mau, UP

Sub-project 2: *Fusarium*

Crop	***Institutes***
Chickpea and Pigeon pea	IIPR, Kanpur, IARI, New Delhi
Safflower	DOR, Hyderabad
Guava	CISH, Lucknow
Banana	NRCB, Thrichy
Tomato, Chilli	IIVR, Varanasi
Biological Control	NBAII, Bangalore
Conservation & characterization	NBAIM, Mau, UP

Sub-project 3: *Ralstonia*

Crop	***Institutes***
Ginger	IISR ,Calicut, PDBC Bengalure
Solanaceous vegetables (Tomato Brinjal, Chilli)	IIHR, Bangalore, IARI, New Delhi, ICAR-RC-Goa, ICAR RC NEH, Umiam, NBAII, Bengalure

The main objectives of the project are

- Diversity study of pathogens *viz. Phytophthora*, *Fusarium* and *Ralstonia*
- Development of diagnostic and detection methodology
- Host-pathogen and microbe interaction studies
- Identification of host resistance using molecular tools
- Development of disease management strategies including IDM and biocontrol agents
- Development of Genomics & Bioinformatics supporting system

PHYTOPHTHORA

Crop wise and detailed reports were presented by various workers from institutions involved.

Indian Institute of Spices Research, Kozhikode
The report on *Phytophthora* disease of Black pepper was presented by Dr K. Nirmal Babu, Principal Scientist, Indian Institute of Spices Research, Kozhikode.

Central Potato Research Institute, Shimla
The report on *Phytophthora* disease of Potato was presented by Dr S.K. Chakrabarty, Head, Plant pathology, Central Potato Research Institute, Shimla.

Central Plantation Crops Research Institute, Kasaragod
The report on *Phytophthora* disease of Coconut and Cocoa was presented by Dr R. Chandramohan, Head, Plant Protection, Central Plantation Crops Research Institute, Kasaragod.

Central Tuber Crops Research Institute, Thiruvananthapuram.
The report on *Phytophthora* disease of Colocasia was presented by Dr S.S.Veena, Senior Scientist, Central Tuber Crops Research Institute, Thiruvananthapuram.

National Research Centre for Citrus, Nagpur
The report on *Phytophthora* disease of Citrus was presented by Dr A.K. Das, Senior Scientist, National Research Centre for Citrus, Nagpur.

ICAR Research Complex for NEH Region, Umaim
Another report on *Phytophthora* disease of Citrus in North Eastern Region was presented by Dr R. Dutta, Senior Scientist, ICAR Research Complex for NEH Region, Umaim.

Dr. Y.S. Parmar University of Horticulture and Forestry, Kullu
The report on *Phytophthora* disease of Apple was presented by Professor I.M. Sharma, Dr. Y.S. Parmar University of Horticulture and Forestry, Kullu.

National Bureau of Agriculturally Important Insects, Benguluru
The report on Biological control of *Phytophthora* using Trichoderma carried out at NBAII, Bengaluru was presented by Dr. S. Sriram.

Executive summary
Phytophthora

Phytophthora species belong to a group of eukaryotic microorganisms classified as oomycetes that are phylogenetically distant from true fungi. Species of the oomycete genus Phytophthora are destructive pathogens, causing extensive losses in agricultural crops and natural ecosystems. Due to their distinct physiological and biochemical characteristics, it is difficult to efficiently control the diseases caused by these pathogens. Current disease control measures are largely dependent on application of chemicals, and novel approaches are urgently needed. It is difficult to control Phytophthora diseases in the tropics because of its wide host range and environmental conditions that are conducive to disease development. Generally the infection goes unnoticed until symptoms like foliar yellowing or wilting appears. Studies are undertaken on seven species of Phytophthora viz. P. cactorum (apple), P. capsici (black pepper), P. citrophthora (citrus), P. colocasiae (taro), P. infestans (potato), P. nicotianae (citrus) and P. palmivora (coconut, cocoa & citrus

Diversity and Distribution

- Occurrence and distribution of major *Phytophthora* diseases of various crops (bud rot-coconut, black pod and stem canker-cocoa, decline-citrus, collar rot-apple and foot rot-black pepper) were recorded and the collected *Phytophthora* isolates from black pepper, potato, citrus, coconut, cocoa and other horticultural and fruit crops have been conserved in the National Repository of *Phytophthora* at IISR, Kozhikode and other research centres viz CPRI, Shimla, NRC for Citrus, Nagpur and CPCRI, Kasaragod.

Colony & sporangial morphology

- Apple, black pepper, citrus, coconut and coaoa isolates were morphologically characterized. The morphological characterization of the *Phytophthora* isolates showed high diversity among them. The *Phytophthora* isolates from black pepper showed eight different types of colony morphology and nine different types of sporangial morphology. *P. palmivora* isolates from coconut showed three different types of colony morphology and two types of sporangial morphology. *P. nicotianae* isolates from citrus showed 11 different colony types on V8 agar and 7 different types on PDA whereas *P. palmivora* isolates showed 3 and 5 patterns in V8 agar and PDA respectively.

Metalaxyl sensitivity

- The metalaxyl sensitivity of *Phytophthora* isolates from black pepper (100 isolates), citrus (37 isolates) and potato was studied using different concentrations of Metalaxyl-mz and Mancozeb and resistant/tolerant isolates were identified. In no case correlation could be observed between metalaxyl resistance and virulence/aggressiveness of the pathogen isolates

Mating types

- Among *P. infestans* the A2 mating type has displaced the A1 population in temperate highlands while in sub-tropical plains, A1 is still dominating. Similarly in citrus out of 119 isolates tested, only 11 isolates were found as A2 mating type and others were of A1 mating type. In case of *P. capsici* and *P. colocasiae* isolates, majority were of A1 mating type. While among 129 *P. palmivora* isolates of coconut, 128 isolates were A2 mating types indicating the predominance of A2 mating type.

Molecular diversity

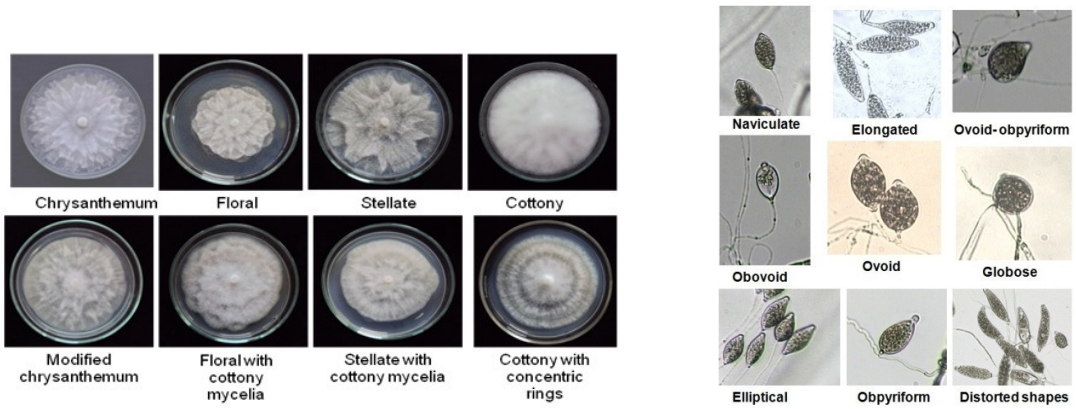
- Genetic diversity and fingerprinting of *Phytophthora* isolates using different molecular markers suggest that *Phytophthora* isolates are at rapid pace of evolution with high level of diversity among isolates.
- ITS-PCR studies in black pepper *Phytophthora* isolates revealed the presence of species like *P. tropicalis*, *P. citophthora, P. nicotianae, P. palmivora* etc. apart from *P. capsici*.
- Studies on mt DNA haplotyping revealed that Indian population of *P. infestans* is composed of Ia and Ib and the population of new mt DNA haplotype Ia is on the rise.
- Diversity analysis of *Phytophthora* associated with citrus using PCR-RFLP analysis of the ITS region (with the restriction enzymes *Msp*I, *Alu*I and *Rsa*I) using ITS 4 and ITS 6 primers revealed the presence of *P. nicotianae, P. palmivora* and *P. citrophthora*. Intra-species variation was observed more in *P. nicotianae* isolates than in *P. palmivora* isolates.
- *P. insolita* (NRCPh- 119) was isolated from water accumulated under the canopy of a Nagpur mandarin tree from Nagpur region for the first time from India.
- Molecular characterization of *Phytophthora* isolates from coconut and cocoa confirmed the association of *P. nicotianae* and *P. capsici* with coconut bud rot and fruit rot, respectively and *P. capsici* to black pod disease of cocoa in addition to the predominant species *P. palmivora*.

Details of *Phytophthora* isolates maintained in repositories

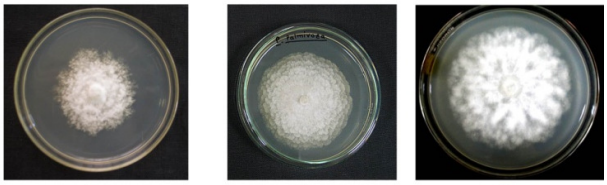
Sl. No	Host plant	No. of isolates	Sl. No.	Host plant	No. of isolates
1	Black pepper	186	20	Piper chaba	04
2	Colocasiae	60	21	Tapioca	03
3	Tomato	09	22	Bauhinia	02
4	Vanilla	03	23	Potato	302
5	Coconut	138	24	Papaya	01
6	Strawberry	03	25	Clove	01
7	Crossandra	02	26	Carnation	01
8	Gerbera	02	27	Vigna	02
9	Perwincle	03	28	Trichosanthes	01
10	Betelvine	24	29	Brinjal	01
11	Cardamom	12	30	Sesamum	01
12	Cocoa	394	31	Avocado	01
13	Rubber	08	32	Yam	01
14	Capsicum	03	33	Diffenbachia	01
15	Nutmeg	03	34	Pineapple	01
16	Citrus	131	35	Apple	114
17	Arecanut	05	36	Geranium	01
18	Piper longum	01	37	Cinnamon	02
19	Plectranthus	01			

Institute	Total collection
IISR	355
CPRI	301
CPCRI	510
CTCRI	50
NRCC	119
YSPUHF	113
TOTAL	**1448**

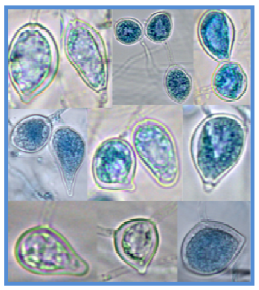
- The AFLP analysis of 15 *P. colocasiae* isolates from taro revealed high level of genetic diversity and grouped the isolates into three major clusters.
- Sequence based haplotype and population genetic studies indicated high variability in black pepper burrowing nematode populations compared to global populations.



Colony and sporangial morphology of black pepper isolates

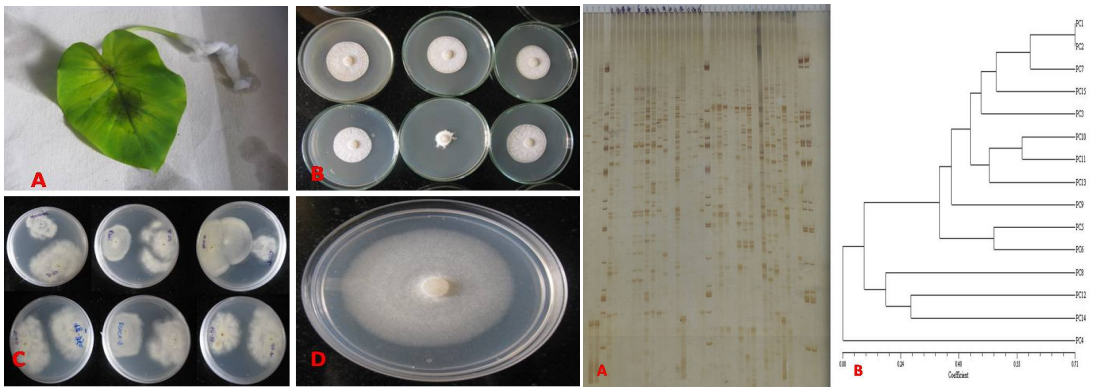


Citrus isolates A. *P. nicotianae* B. *P. palmivora* C. *P. citrophthora*



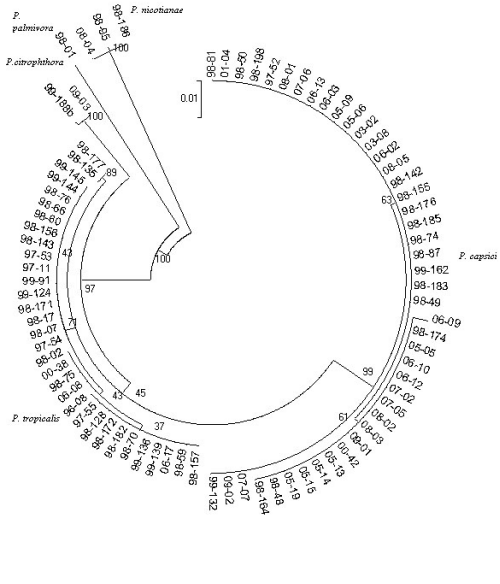
Sporangial variations of the different isolates of *Phytophthora* from Northeast India

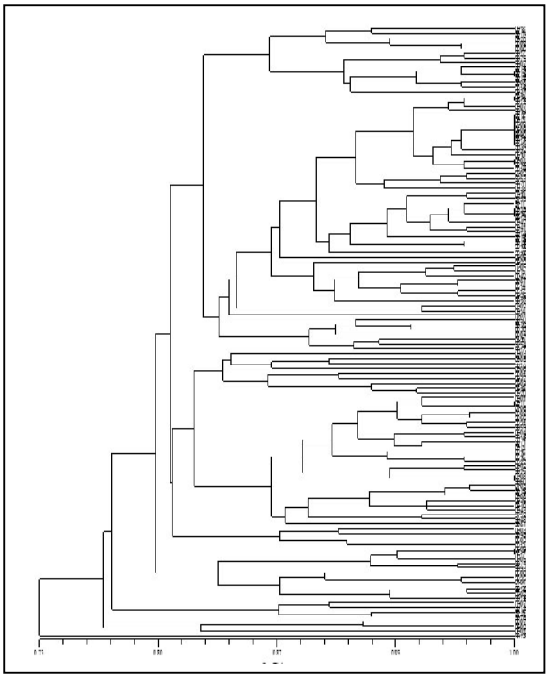
Virulence and molecular diversity



A: Virulence assay on detached taro leaf, B: Metalaxyl sensitivity (0, 0.1, 1, 5, 10, and 100µg ml^{-1}; Isolate: PC 16.) C: Mating type, D: Media characterization

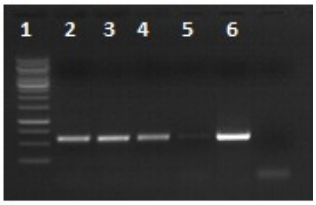
A: Urea-PAGE gel, B: Dendrogram depicting the genetic relatedness of *P. colocasiae* by AFLP analysis



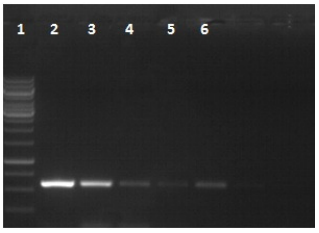


Dendrogram depicting high diversity among 126 *Phytophthora* isolates from black pepper

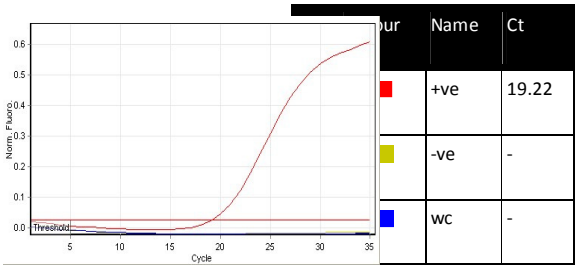
Diagnostics



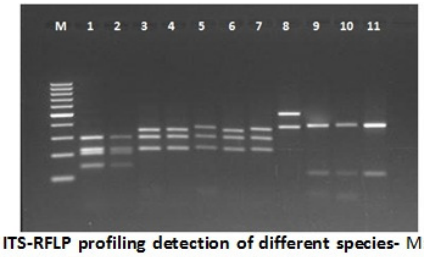
Detection of *P. infestans* from diseased tissue.
1=Ladder, 2= Symptomatic tissue, Healthy tissue (3= 5 mm away, 4= 10 mm away, 5= 20mm away), 6= DNA of *P. infestans,* 7= Control (un-inoculated tuber)



Detection of P. infestans.
1=Ladder, 2= 1ng/ul, 3= 100pg/ul, 4= 10pg/ul 5= 1pg/ul, 6= 100fg/ul, 7= 10fg/ul (very faint band)



Real time PCR detection of *P. capsici*



ITS-RFLP profiling detection of different species- M: 100bp ladder, Lane 1&2: *P. capsici,* Lane 3,4,6&7: *P. tropicalis,* Lane 5: *P. citrophthora,* Lane 8: *P. palmivora,* Lane 9,10 &11: *P. nicotianae*

- PCR detection of *P. capsici* from infested black pepper soil using CAPFw and CAPRv2 primers was standardized.
- For detection of latent infection of *P. infestans* in host tissues, a PCR protocol was standardized with a sensitivity to detect 10 ng of genomic DNA and up to 20 mm away from the diseased tissues.
- New methods for detection of *P. nicotianae* and *P. palmivora* in citrus roots and rhizospheric soils and water using nested PCR and PCR-RFLP technique have been developed.
- New sets of species specific primers were designed using the ITS sequence data for detecting burrowing nematodes from black pepper rhizosphere soil.
- Real time PCR detection of *P. capsici* using primers designed from RAPD-SCAR region have been standardized.
  Diagnotistic profiles for detection of five species *viz. P. capsici, P. tropicalis, P. palmivora, P. nicotianae* and *P. citrophthora* using ITS-RFLP have been developed

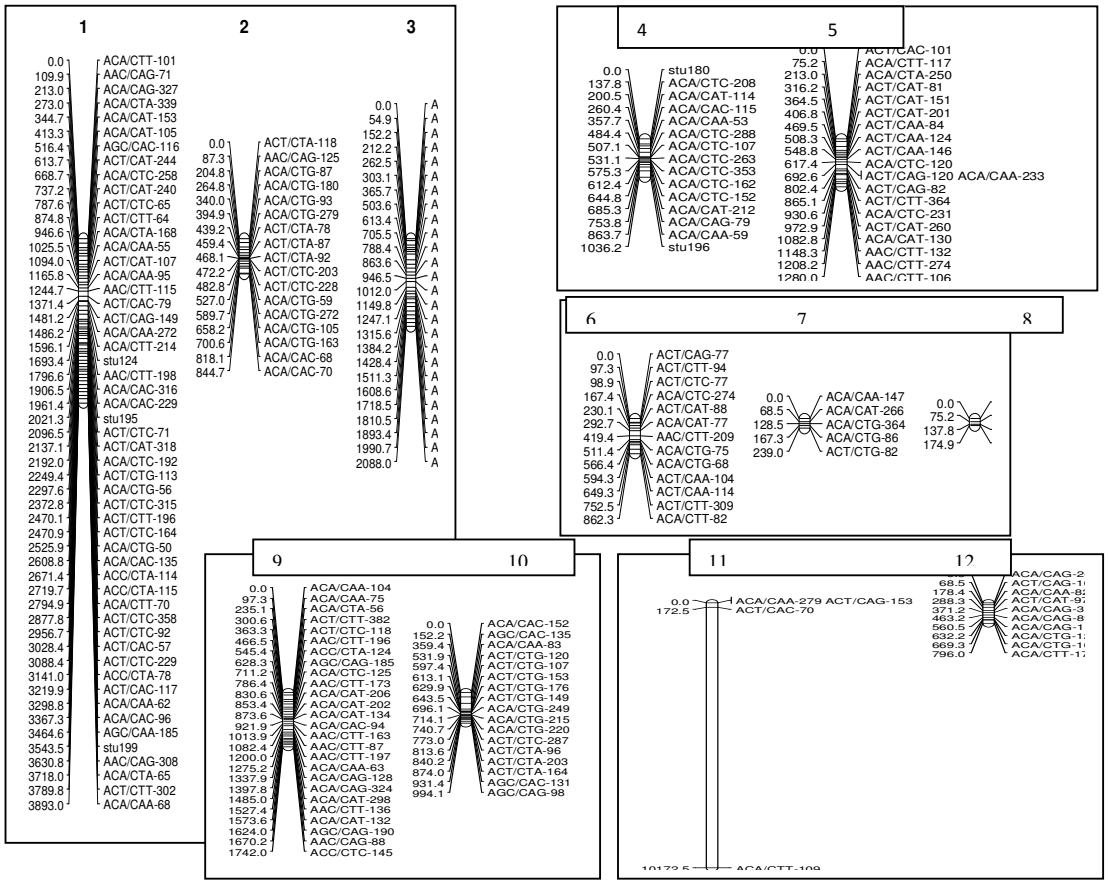
Host-Plant resistance

Resistant / tolerant lines identified

Crop	Promising lines
Apple	M9, M26, *Malus prunifolia* and *Malus floribunda* (rootstocks) and Vance Delicious (cultivar) highly resistant against *P. cactorum*
Black pepper	Acc. No. 1324 (Aimpiriyan) and HP 780 (Perambramundi X Karimunda)
Citrus	Rough lemon x Trifoliate hybrid and Troyer citrange (Chethali) identified as moderately tolerant to *Phytophthora* root rot
Cocoa	Accession collected from Thrissur, Kerala

Molecular map

- Using molecular markers linked to late blight resistance genes, R1 gene was confirmed to be present in 23 potato genotypes whereas 61 possessed R3a gene. Attempts for gene pyramiding using molecular assisted selection were made.
- Molecular mapping of quantitative trait loci (QTL) for horizontal resistance to late blight in the diploid potato species *Solanum chacoense.* The molecular linkage map of *S. chacoense* was prepared with a total of 208 AFLP markers.



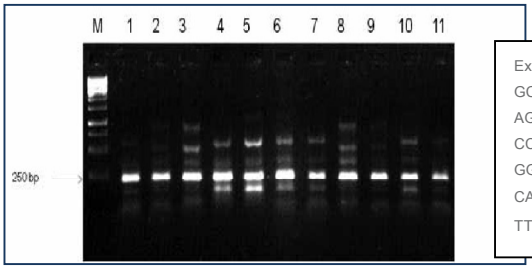
Molecular linkage map of *S. chacoense*

- *Phytophthora Avr*3a a virulence gene having RXLR motif was identified for siRNA and amiRNA mediated gene silencing for late blight resistance development in potato. Two potato cultivars (Kufri Khyati and Kufri Pukhraj) were transformed with siRNA and amiRNA gene constructs.

Putative transformants were multiplied *in-vitro* and all positive lines of siRNA and amiRNA transgenic plants were multiplied under *in-vitro* condition for further screening.

Potato transformation with *Avr3a* RNAi gene constructs iIR-*Avr3a*.

- R genes and gene analogues were amplified, cloned and sequenced from resistant sources of black pepper using degenerate primers.
- The elicitin gene was amplified from *P. capsici* and wrky gene from *Piper Colubrinum* using custom designed primers.



Expected Size 220bp

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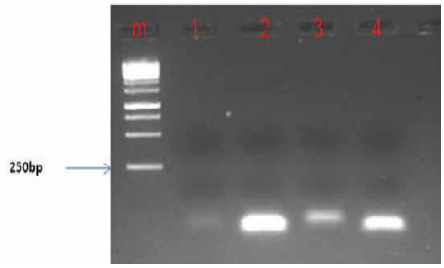
CCTGACTGCGAGCTGACGGTGCCCACGAGCGGTCTGGTGCTCAACGTTAACTCGTACGCGAAC
GGGTTCTCGACGAGTCCGAGAGGATCCTACTGTTCCGAGATGAGCTACATGGTTCGACGGC
CAACGGGGCCCACATTTCCTTGATTCAGAGCAGATCGTGGGCAAGTTGAAAAAATGCCCAAAAG
TTTTAAAA

Amplification and sequencing of elicitin gene from *P. capsici*



Expected Size 143 bp

CGATTCGAGAGTTGATAGACTACGTTGCTTATTATCCTCCTGAGTATGACCCCGAGACAGTGA
CACCTTGCCAACATACGAGTACTGCTCCTCCTGGAGTAAAACTGAGCTTCCACGTCCTGCAG
TGCTGAACTTCCATCTTCGGTCATGAACCTCGCCGGATCGACTACCCCGCGAGTCACCCGCT
GTCCAAAATAGGTTGTTACAACAAAGAGCCTGTTGCTGGAGAAAAAATCCACATATTTGTAAT
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AACGTATCCGGGTTCAAAGCTCTGTTGCTCCACGTTTGGAAGATTTGCGAATCCCTGTTG
TATGAAAAACTTCCCAGGACCGCCTCACGGTATCCATGTCGAGAGAGAAAATTTACACATGTAT
GCTCCCCTCTATTGAGAAGCATAAAAAACCTAAATTGGCGTTGTCTGCCAAAACTACCGAC
AGCAGTTTATGATGTCTTCTGGTGATTTTCTTAAAAAC

Amplification and sequencing of wrky gene (143 bp) from *Piper colubrinum*

- In black pepper the association mapping population comprising of 57 genotypes was molecular characterized for tagging *Phytophthora* resistance genes.
- Attempts to characterize the putative NBS-LRR regions of leaf blight resistant taro cv. Muktakeshi were made using primers designed from conserved sequence motifs.

Epidemiology and Disease management

Development of Decision Support System (DSS) for late blight management in potato

Developed Decision Support System for western Uttar Pradesh which has three components i.e. decision rules for prediction of first appearance of late blight in potato, decision rules for need based fungicide application, and yield loss assessment model.

Epedemialogy

Spread of bud rot in coconut

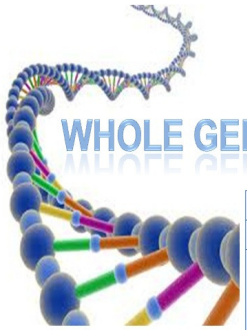
Retention of affected coconut palms and slugs (*Deroceras* sps.) were found to be a major source of inoculum and spread of bud rot disease in coconut.

Integrated disease management strategies developed

Collar rot of Apple	Combined applications *T. harzianum* -TH 15 (200g bran culture + 50 g talc formulation), *Enterobacter aerogenes* –EA2 (200 g coconut coir culture + 50 g talc formulation) in the first week of April and last week of August, biofumigation with mustard plants (first week of March) and metalaxyl MZ @ 0.3% (April and August)
Bud rot of coconut	Use of Mancozeb and phosphorous acid and an organic formulation of *Trichoderma* will help in managing bud rot disease of coconut.. A slow release fungicide in the form of sachets was developed for dispersal of mancozeb to the coconut crown.
Foot rot of black pepper	An IDM strategy has been established for Black pepper using endophytic bacteria *Curtobacterium luteum* (TC 10) as root treatment at the time of planting followed by soil application of the same and Metalaxyl- mz twice during the monsoon season.
Leaf blight in taro	An effective isolate *T. harzianum* was formulated in Talc with wheat bran (5:1). Isolates of *Trichoderma* spp. that elicit induced systemic resistance in terms of phenol production and enhanced activities of peroxidase, polyphenol oxidase and glucanase were identified.
Stem canker of cocoa	Phosphorus acid and *T. harzianum* were found to be better in controlling stem canker of cocoa

List of promising bio-control agents identified

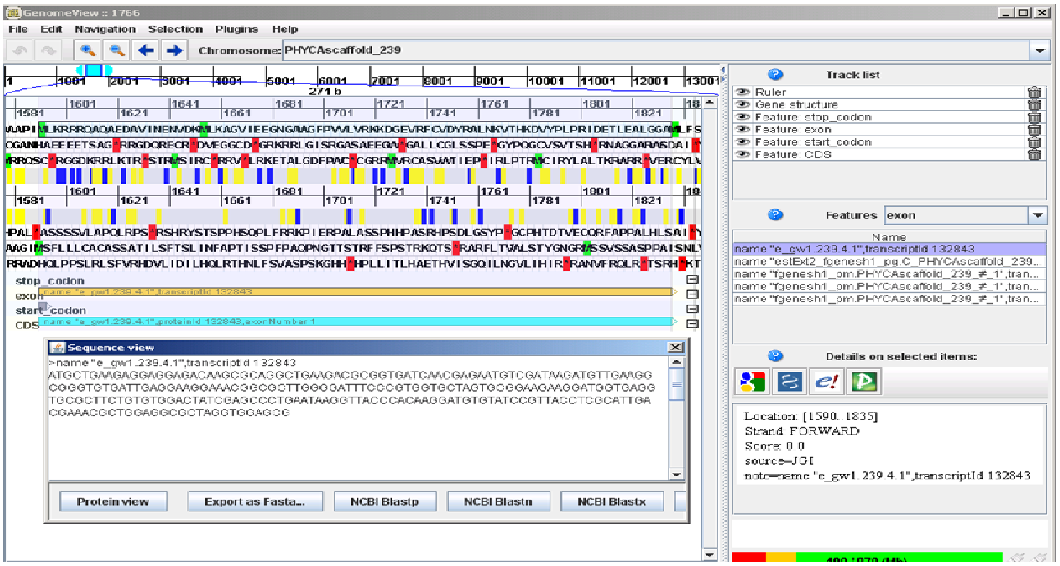
Institute	**Crop/Disease**	**Biocontrol agent**
IISR, Calicut	Black pepper-*Phytophthora* foot rot	**Endophytic bacteria**- *Curtobacterium luteum, Bacillus megaterium, P. putida* **Endophytic fungi** - *Annulohypoxylon nitens*, *Fusarium proliferatum, Daldinia eschscholzii, Gibberella moniliformis and Ceriporia lacerate* **Actinomycetes**- *Streptomyces* sp-(Act 7)
NRC Citrus	Citrus - *Phytophthora*	*Trichoderma* spp, NRCfBA-44 and NRCfBA –29 (*T. harzianum*) *Trichoderma,* PF-6 and PF-11
ICAR RC for NEH	Citrus - *Phytophthora*	*Trichoderma brevicompactum, T. harzianum, T. longibrachiatum*
CPCRI, Kasargod	Cocoa - stem canker	*T. harzianum*
CPCRI, Kasargod	Coconut - bud rot	*T. harzianum*
CTCRI, Trivandrum	Colocasia-leaf blight	*T. harzianum* (T7)
YSPUHF Kullu	Apple	*T. harzianum* -TH 15 *Enterobacter aerogenes* –EA2
NBIAM		Isolates of *Trichoderma* spp. that elicit induced systemic resistance in terms of phenol production and enhanced activities of peroxidase, polyphenol oxidase and glucanase were identified.



Total number of base pairs	64.05 Mb	
Total number of reads	2.26 million	
Base composition	A	23.04%
	C	26.43%
	G	27.13%
	T	23.40%
Total number of scaffolds	917	
Size of the largest scaffold	21,709,55 bp	
Size of the smallest scaffold	1001 bp	
Total number of SNPs	3,304,10	
Total number of Indels	2,404,24	

A native isolate of *Phytophthora capsici*, (Is. No. 98-93) infecting black pepper was completely sequenced using next generation sequencing platform, Illumina - Solexa GA II. The sequence data was assembled by taking Joint Genome Institute's *P. capsici* as reference genome with ~ 87.53 % coverage.

Genome view



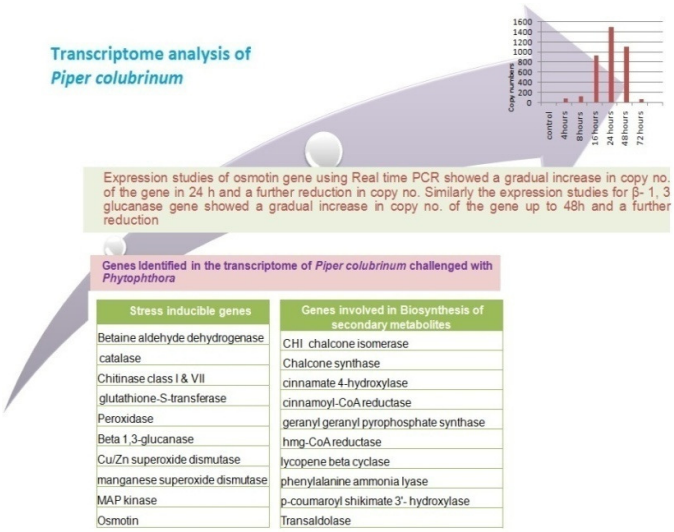
Comparison of *P. capsici* of IISR strain with *P. capsici* (JGI), *P. infestans*, *P. ramorum*, *P. sojae*

Organism	Number of scaffolds	Nucleotide composition				Genome Size (Mb)	Number of genes	Percentage Identity with others
		A	C	G	T			
P. capsici (IISR)	917	23.04%	26.43%	27.13%	23.40%	64.05	19,805	
P. capsici (JGI)	917	23.04%	26.43%	27.13%	23.40%	64	19,805	40%
P. infestans	4921	25.11%	23.39%	26.10%	25.40%	240	22658	0.9%
P. sojae	1810	22.77%	27.25%	27.20%	22.78%	95	19027	0.6%
p. ramorum	2576	25.96%	23.94%	23.99%	26.11%	65	15743	0.009%

Host-pathogen interaction studies: identification and analysis of genes expressed under stress

Transcriptome analysis in Piper

Transcriptome analysis was performed in *Piper* to identify, characterize and catalogue all the transcripts expressed involved in *Piper* – *Phytophthora* interactions.A variety of genes viz., stress induced, related to secondary metabolism, transcription factors and involved in primary metabolism with significant similarity to those characterized in other plants were identified.



Summary statistics	*Piper colubrinum*	*Piper nigrum*
Sequence File Size	37.70 MB	76.06 MB
Maximum Sequence Length	15769	10479
Minimum Sequence Length	100	100
Average Sequence Length	567.844	721.922
No. of Sequences	62619	101284
Total Sequences Length	35557875	73119148
Total Number of Non-ATGC Characters	1316	1090
Percentage of Non-ATGC Characters	0.00004	0.00001

EST annotation

- *Phytophthora capsici* EST assembly and annotation has revealed that 84.73% of the ESTs displayed significant similarity to known sequences in GenBank.
- Extracellular effector proteins from *Phytophthora capsici* were predicted through EST mining and secretome analysis.

Phytoweb, a comprehensive portal on *Phytophthora* diseases of horticultural crops in India was developed by modifying the exisiting PhyDisH. This portal has two components, a public portal on various *Phytophthora* diseases of horticultural crops, their management methods and a catalogue of genotypic and phenotypic data on *Phytophthora* cultures maintained in the repository. The second component is a web interface for monitoring the PhytoFuRa project on a real time basis. All participating institutes can login to the system and can submit their periodic progress reports and financial statements etc. The project leaders and policy makers can view the compiled periodic progress reports, financial statements etc.

Phytophthora Genome Database that provides access to primary structure of the *Phytophthora* genome including genome sequence, number of genes, CDS, SNPs, InDels, nucleotide composition, intron-exon structure, start and stop codon, intron lengths, alternative splicing and untranslated regions (UTRs) was developed.

FUSARIUM

Crop wise and detailed reports were presented by various workers from institutions involved.

Indian Agricultural Research Institute, New Delhi
The report on *Fusarium wilt of* Chick pea was presented by Dr S.C. Dubey, Principal Scientist, Indian Agricultural Research Institute, New Delhi.

Indian Institute of Pulses Research, Kanpur
Another report on *Fusarium* wilt of Chick pea and Pigeon pea was presented by Dr R.G. Choudary, Principal Scientist, Indian Institute of Pulses Research, Kanpur.

Directorate of Oilseeds Research, Hyderabad
The report on *Fusarium* wilt of Safflower was presented by Dr R.D. Prasad, Senoirl Scientist, Directorate of Oilseeds Research, Hyderabad.

Central Institute of Subtropical Horticulture, Lucknow
The report on *Fusarium* wilt of Guava was presented by Dr B.K. Pandey, Principal Scientist, Central Institute of Subtropical Horticulture, Lucknow .

Indian Institute of Vegetable Research, Varanasi
The report on *Fusarium* wilt of Tomato and Chilli was presented by Dr M. Loganathan, Senior Scientist, Indian Institute of Vegetable Research, Varanasi.

National Research Centre for Banana, Thiruchirapalli
The report on *Fusarium* wilt of Banana was presented by Dr R. Thangavelu, Senior Scientist, National Research Centre for Banana, Thiruchirapalli.

National Bureau of Agriculturally Important Insects, Bangalore
The report on biological control of *Fusarium was* presented by Dr S. Sriram, Senior Scientist, National Bureau of Agriculturally Important Insects, Bangaluru.

National Bureau of Agriculturally Important Micro-organisms, Mau
The report on conservation and characterization of *Fusarium* at the National Bureau of Agriculturally Important Micro-organisms, Mau was presented by Dr Sudheer Kumar, Senior Scientist from Mau.

Executive summary

Fusarium

Wilt caused by species of Fusarium is one of the most serious disease problems of several agricultural, vegetable and fruit crops. Since, Annual yield losses severity up to 10% in chickpea, 97000 t in pigeon pea, 25% each in safflower and chilli and 30% in banana have been reported depending upon the disease severity and crop stage. One control method is to improve soil conditions because Fusarium spreads faster through soils that have high moisture and bad drainage. Other methods include planting resistant varieties, removing infected plant tissue to prevent overwintering of the disease, using soil and systemic fungicides to eradicate the disease from the soil, flood fallowing, and using clean seeds each year. Applying fungicides depends on the field environment. The species studied are F. oxysporum f. sp. carthami (safflower), F. oxysporum f. sp. ciceris (chickpea), F. oxysporum f.sp. cubense (banana), F. oxysporum f. sp. lycopersici (tomato), F. oxysporum f. sp. psidii (guava), F. solani (chilli) and F. udum (pigeon pea).

Diversity

Surveys were conducted in banana, chilli, chick pea, guava, pigeon pea, safflower, tomato etc and collected several isolates of *Fusarium viz*. *Fusarium oxysporum* f. sp. *cubense* (*Foc). F. udum, F.oxysporum* f.sp. *ciceri, Fusarium oxysporum* f. sp. *ciceris , Fusarium oxysporum* f. sp. *lycopersici, F. solani etc.*

Institute	Crop	Pathogen	No. of isolates
CISH, Lucknow	Guava	*F. oxysporum* f. sp. *psidii*	154
DoR	Safflower	*Fusarium oxysporum* f. sp. *carthami*,	54
IIVR, Varanasi	Tomato Chilli	*F. oxysporum* f. sp. *lycopersici* *F. solani*	105 124
IIPR, Kanpur	Chickpea Pigeon pea	*F.oxysporum* f.sp. *ciceri,* *F. udum,*	70 40
NRC Banana	Banana	*F. oxysporum* f.sp. *cubense*	180

Distribution of different races of *Fusarium* sp.in India

Five variants of *F. udum* and seven races of *F. oxysporum* f.sp. *ciceri* were identified and their distribution in different states of India was documented.

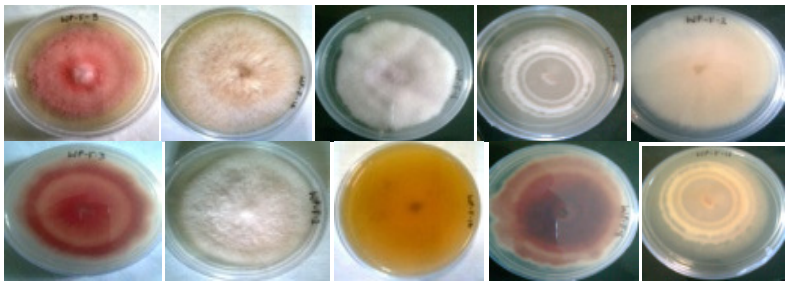
States	Variants of *F.udum*	Races of *F. oxysporum* f.sp. *ciceri*
Andhra Pradesh	1, 2	2, 6
Bihar	2, 3	-
Chandigarh	-	3
Delhi	-	3
Gujarat	-	0, 3
Haryana	1	2, 3, 4
Jharkhand	2, 4	3, 5
Karnataka	1, 2, 3, 5	1, 3, 4, 5
M.P.	1, 2, 3	1, 2, 3, 6
Maharastra	1, 2, 4	3
Punjab	-	3, 5
Rajasthan	1	0, 3, 4, 5, 6
Tamil nadu	1, 2	-
West Bengal	4	-
U.P.	1, 2, 3, 4, 5	0, 2, 3, 4, 5

Different isolates of *Fusarium* conserved for long term at NBAIM, Mau

Institute	Crop	*Fusarium* spp.	Cultures	Accession Numbers
IIPR, Kanpur	Chickpea	*F. udum*	20	NAIMCC-F-02862 - NAIMCC-F-02881
	Pigeon pea	*F. o.* f. sp. *ciceri*	20	NAIMCC-F-02842 - NAIMCC-F-02861
IIVR, Varanasi	Tomato	*F. o.* f.sp. *lycopersici*	28	NAIMCC-F-02780 - NAIMCC-F-02807
	Chilli	*F. solani*	34	NAIMCC-F-02808 - NAIMCC-F-02841
NBAII, Bangalore	Tomato and chilli	*F. solani*	4	NAIMCC-F-02970 - NAIMCC-F-02973

Morphologivcal charecterisation and Colony morphology

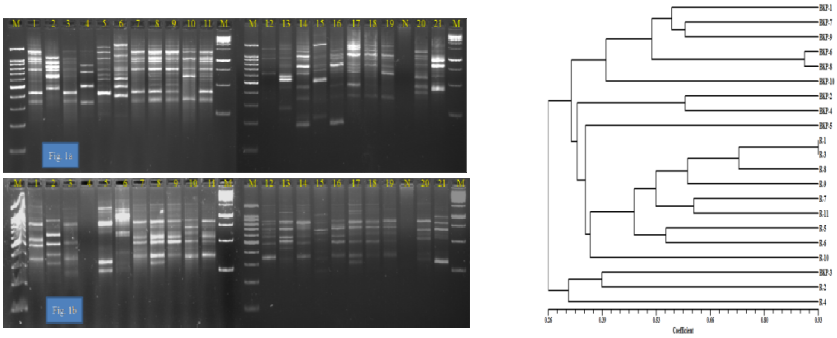
The isolates of *F. oxysporum* were characterized for morphological variability on the basis of pigmentation, growth pattern, colony colour, mycelia colour, shape and size of micro conidia and macro conidia etc



Variation in colony and pigmentation in different isolates of *F. oxysporum* f. sp. lycopersici

Molecular characterization

Genetic diversity in *Fusarium* isolates was analyzed using molecular markers like RAPD (*Fusarium oxysporum* f. sp. *carthami, F. oxysporum* f. sp. *psidii, F.oxysporum* f.sp. *ciceri, F. oxysporum* f.sp. *lycopersici*), ISSR (*F. oxysporum* f.sp. *cubense, F.oxysporum* f.sp. *ciceri*), ITS, TEF-1α), *β*-tubulin (*Fusarium oxysporum* f. sp. *ciceris*), SSR (*F.oxysporum* f.sp. *ciceri, Fusarium udum*)



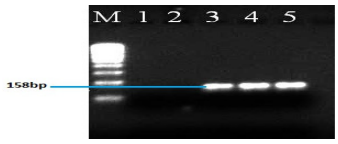
RAPD profiling (Fig. 1a&1b) and dendrogram of 21 *F. oxysporum* f. sp. *psidii* isolates derived from RAPD fingerprints generated by UPGMA

Host resistance

Fusarium Resistant/tolerant lines identified in different crops

Crop	Promising lines
Safflower	Hybrids of *C. tinctorius* x *C. glaucus*, C. *tinctoriusx* C. *oxyacantha*, *C. tinctorius* x *C tinctorius*, *C. tinctorius* x *C. turkestanicus*, *C. tinctorius* x *C. lanatus* and C. *tinctorius* x *C. creticus*
Pigeon pea	AWR 74/15, BDN 1, Banda Palera, MA 3, ICP 8858, ICP 8859, ICP8863, ICP 9174, KPL 43, KPL 44, PI 397430, IPF 9 and IPA 38
Chickpea	IPC nos. 2004-3, 2004-8, 2004-34, 2004-52, 2005-15, 2005-19, 2005-24 and KGD 1255
Guava	Hybrid, *Psidium molle x P. guajava*
Tomato	BTH-9 (M), Indam 2102-10-1, Indam-2103-1-2, A-15-6-1, EC-620381, A-15-9-1, IIVR-61, IIVR-40 and IIVR-28
Chilli	Local colle.-35, PBC-904-UP, CV-1, BS-5, COO-713, COO-304, PDC-24, IC-383072 and LCA-335

Diagnostics

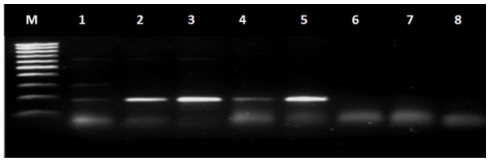


In planta detection of Foc by SCAR marker. M – Marker (100 bp) 1. Control (without infection) 2. Root 3. Corm 4. Pseudostem

A SCAR marker was developed for identifying *Fusarium oxysporum* f. sp. *carthami, F. oxysporum* f. sp. *ciceris* and *F. oxysporum* f.sp. *cubense* from other species of *Fusarium* based on the ITS sequence.

Identified RAPD marker linked to *Fusarium* wilt resistance in *C. tinctorius.*

Species specific primers were designed for specific detection of *F.oxysporum* f.sp. *ciceri, Fusarium oxysporum* f. sp. *psidii*.



PCR amplification of ITS region with specific primer BKP-1/BKP-2, Lane 1 – 5: *F. oxysporum* f. sp. *psidii* isolates, Lane 6: *F. oxysporum* f. sp. *cubense*, Lane 7: *F. oxysporum* f. sp. *ciceris*, Lane 8: *F. moniliformae*, M: 100 bp DNA Ruler.

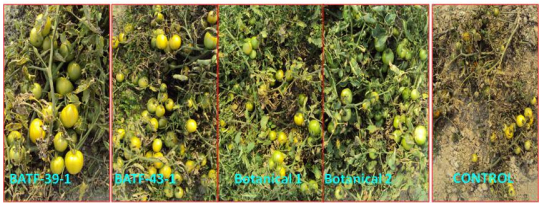
A multiplex PCR and a colony PCR assays were developed for identification of *F. oxysporum* f. sp. *psidii*.

Disease management

Promising biocontrol agents

Disease	Bio control agent
Fusarium wilt of pigeon pea Fusarium wilt of chickpea	*Trichoderma viride* (Kanpur) *Trichoderma* strains 1,2,3,4,12,13,14
Safflower wilt	*Trichoderma harzianum*, Th4d
Banana	*Trichoderma harzianum* non-pathogenic *Fusarium*
Tomato & chilli	*Trichoderma spp.*

Difenaconazole +*T. aperellum treatment*



Effect of *Trichoderma* isolates (BAT-39-1 and BATF 43-1) and botanical extract on wilt of tomato under field conditions

- Soil application of Difenaconazole (0.1%) with promising biocontrol agents as well as combined application of different biocontrol agents recorded complete control of Fusarium wilt of banana.
- Dipping of banana plants + soil drench at @ 250 ml/ pot with the leaf extract of *Alpinia galanga* and *Vitex negundo* and Zimmu individually, recorded 100% reduction of *Fusarium* wilt disease compared to control.
- Carboxin, thiophanate-methyl, tetramethyl thiuram disulphide, metalaxyl + mancozeb, captan and mancozeb proved to be compatible with *Trichoderma harzianum*.
- A combination of Pusa 5SD (*T. harzianum*), *P. fluorescens* (Pf-80), *Mesorhizobium ciceri* and vitavax power as seed treatment provided the highest germination and the lowest wilt incidence in chickpea.
- Identified effective IDM components under field conditions: Two, *Trichoderma* isolates *viz.,* BATF-39-1 and BATF-43-1 and 2 botanical extracts were effective in reducing the wilt incidence in chilli and tomato under field conditions

RALSTONIA

Crop wise and detailed reports were presented by various workers from institutions involved.

Indian Institute of Spices Research, Kozhikode
The report on *Ralstonia* (bacterial) wilt of Ginger was presented by Dr R Suseela Bhai, Senior Scientist, Indian Institute of Spices Research, Kozhikode.

ICAR Research Complex for NEH Region, Umaim
The report on *Ralstonia* (bacterial) wilt of Tomato, Brinjal and Chilli was presented by Dr Ram Dutta, Senior Scientist, ICAR Research Complex for NEH Region, Umaim.

Indian Agricultural Research Institute, New Delhi
The report on *Ralstonia* (bacterial) wilt of Tomato and Chilli was presented by Dr Dinesh Singh, Senior Scientist, Indian Agricultural Research Institute, New Delhi.

Indian Institute of Horticultural Research, Benguluru
The second report on *Ralstonia* (bacterial) wilt of Tomato, Brinjal and Chilli was presented by Dr C Gopalakrishnan, Principal Scientist, Indian Institute of Horticultural Research, Benguluru

ICAR Research Complex, Goa
The third report on *Ralstonia* (bacterial) wilt of Tomato, Brinjal and Chilli was presented by Dr M. Thangam, Senior Scientist, ICAR Research Complex, Goa

National Bureau of Agriculturally Important Insects, Benguluru
The report on biological control of *Ralstonia* was presented by Dr S. Sriram, Senior Scientist, National Bureau of Agriculturally Important Insects, Bangaluru.

Executive summary
Ralstonia
Bacterial wilt caused by Ralstonia solanacearum is an important soil-borne disease that spreads worldwide. It belongs to the β-proteobacteria and is considered a "species complex". It has an unusually broad host range which comprises over 200 plant species, representing over 50 botanical families and covering both monocots and dicots extending from annual plants to trees and shrubs. The pathogen has a wide geographical distribution especially in tropical, subtropical, and some temperate regions. It has effective pathogenicity determinants to invade and colonize host plants but, also exhibits successful strategies for survival in harsh conditions. Under PhytoFuRa, bacterial wilt problems of ginger and vegetables are intensively studied.

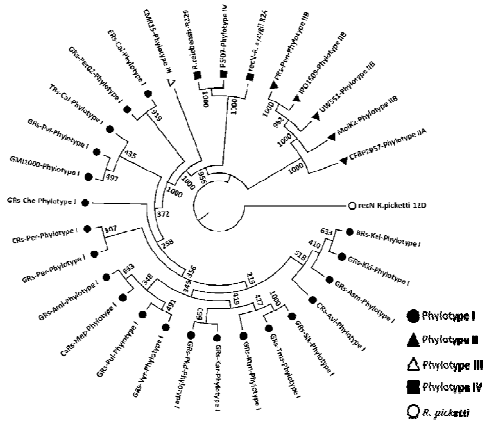
Diversity
A collection of *Ralstonia solanacearum* isolates of representing diverse crops species such as tomato, chilli, eggplant, marigold, ginger and potato were made and conserved. These isolates were characterized for various phenotypes such as pathogenicity on their respective hosts, and biovar.

Institute	No of isolates	Crops
IISR, Kozhikode	30	Ginger, Small Cardamom
IIHR, Bangalore	174	Tomato,,Pepper,,eggplant
IARI, New Delhi	146	Tomato,,Capsicum,,Chilli,,Eggplant,,Potato
ICAR RC Goa	233	Tomato,,Capsicum,,Chilli,,Eggplant,,Marigold,,Sunflower

- Survey on bacterial wilt of tomato, chilli, capsicum, brinjal and potato caused by *Ralstonia solanacearum* was undertaken in disease prone area of Uttarakhand Himachal Pradesh, Jammu &

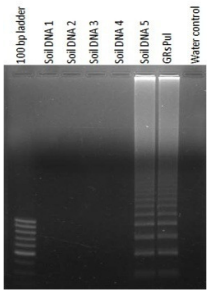
Kashmir, Jharkhand, West Bengal and Orissa. The disease incidence in tomato was quite low (1- 3%) in the summer season in the states of Jharkhand, Uttaranchal, Jammu & Kashmir whereas, it is higher in the rainy season (4 - 60%) in Himachal Pradesh, Jharkhand, Uttarakhand and West Bengal.

- Molecular methods for diversity analysis were standardized based on conserved gene sequences (16s rDNA, egl gene), *recN,* DNA repair protein and intergenic sequences (ERIC). Multilocus Sequence Typing (MLST) and rare cutting pulsed field gel electrophoresis (RC PFGE) were also standardized for the diversity analysis. The results indicated that the bacterium displayed clear genomic diversity among the locations and crop origin.
- Based on C utilization studies, 95% of *R. solanacearum* isolates from solanaceous crops in six states belong to Biovar 3. Multiplex –PCR analysis has shown that all the biovar 3 & 4 isolates of *R. solanacearum* belong to phylotype I.
- Phylotype I biovar 3 strains could be clustered into diverse pulsotypes representing the clonal lines of the *R. solanacearum* species complex by employing rare cutting pulsed field gel electrophoresis (RC PFGE).
- Multilocus Sequence Typing (MLST) using five housekeeping genes (*pps*A, *adk*, *gap*A, *gdh*A, *gyr*B) & three virulence genes (*hrp*B, *fli*C and *egl*) was used for the diversity analysis of 21 strains of *Ralstonia solanacearum* representing different hosts and geographical locations in India. Several novel alleles could be found in different strains of *Ralstonia solanacearum* using this study.
- *rec*N sequence based phylogeny of *R. solanacearum* was in perfect congruence with phylotyping which in turn matches with phenotypic and molecular typing schemes indicating its resolving potential at sub species level



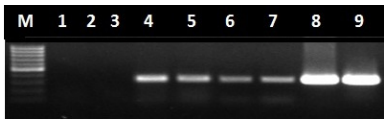
Diversity analysis of ralstonia based on conserved gene sequences (16s rDNA, egl gene), *recN,* DNA repair protein and intergenic sequences (ERIC).

Diagnostics



Loop mediated isothermal amplification for detection of *R. solanacearum* from soil

- *R. solanacearum* could be detected from soil DNA by Loop mediated isothermal amplification (LAMP).
- PCR based detection of Ralstonia **in soil/host tissue** was done using RS specific primer pair which amplifies 0.3kb DNA fragment from bacteria infected sample but not from other samples.
- An *Hrp* gene based marker was developed and validated for detection of *R.solanacearum.*
- Bio-PCR was standardized using 759/760 primer pairs and could detect *R. solanacearum* from infested soil without isolating the DNA.

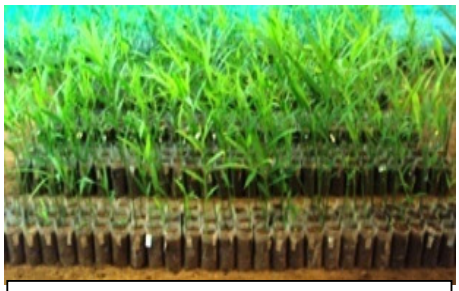


Detection of *R. solanacearum* from irrigated water in farmer' s tomato field by *hrp* gene based a set of primer (Hrp_rs2F and Hrp_rs2R) amplified at 323 bp.

Host resistance

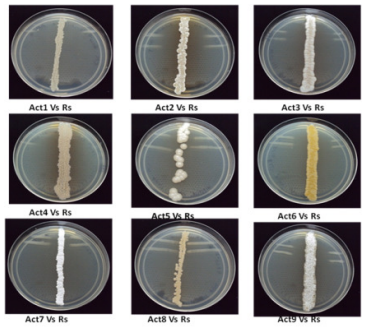
Promising lines identified

Institute	Crop	Total lines screened	Promising lines
IISR, Kozhikode	Ginger	300	5
IIHR, Bangalore	Tomato	39	11
	Brinjal	28	9
IARI, New Delhi	Tomato	13	1



Screening of Ginger germplasm

Disease management



Promising Actinomycetes against *Ralstonia*

Testing of promising biocontrol agents

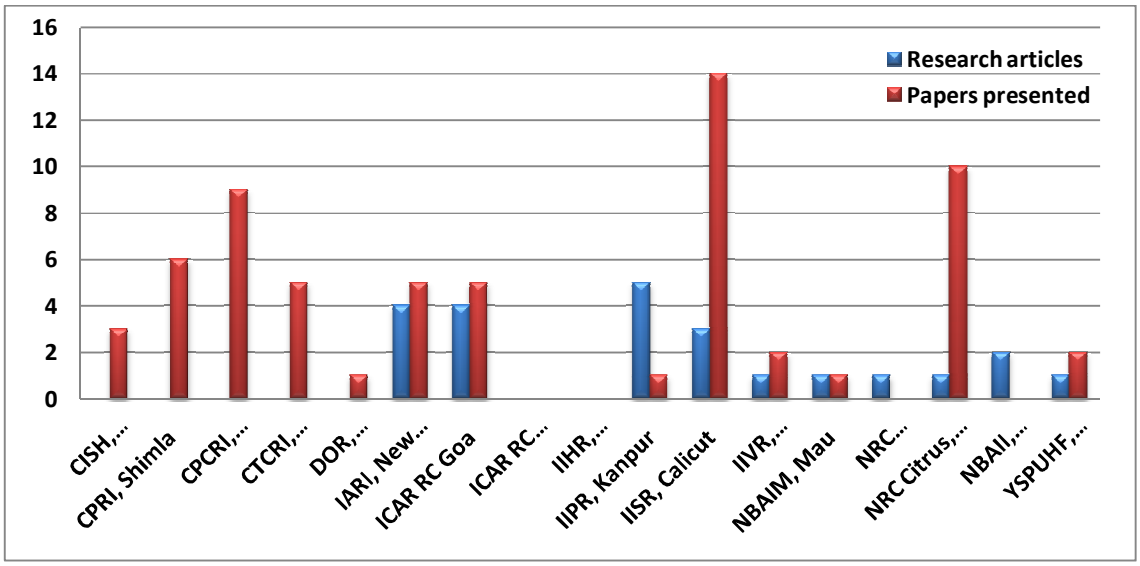
- Xylem residing bacteria was isolated and the isolates are being screened for their antagonism to *R. solanacearum*. The three promising biocontrol agents viz., *P. aeruginosa* (EB69, Rs-08-72) and *Bacillus* spp (EC13) recorded less disease and higher yield in brinjan under field condition.
- Integrated disease management, FYM, green manure and *Pseudomonas fluorescens* (seed treatment) was found to be highly effective in reducing the wilt incidence and increasing the yield in tomato.
- Phages isolated from different locations were found effective against the bacterial wilt pathogen. Four DAPG producing *Pseudomonas* isolates have been identified as potential bioagents against the bacterial wilt pathogen.

BUDGET UTILIZATION

The coordinator requested all the PI s to judiciously spend the balance money and send the complete utilization certificate in time. In case of unspent mount if any this must b informed sufficiently early to the coordinator so that this amount can be utilized by another needy center. He also informed that the final phase of installment is yet to be released by ICAR and it will be distributed as soon as it comes.

PUBLICATIONS

Many research papers were published in this project (See Annexure 1) for details



INFRASTRUCTURE FACILITIES BUILT

The following high end equipment were installed in addition to many routine mol biology facilities

Central Molecular Biology Facility:	IISR
Real time PCR:	IARI, IISR, NRCC, NRCB
Tangential flow filtration unit for purification and mass production of bacteriophages:	NBAII

HRD

A short-term training on 'Application of Genomics and Bioinformatics in *Phytophthora*/*Ralstonia* Research' has been organized exclusively for the project staff of PhytoFuRa at Indian Institute of Spices Research, Calicut from 08-17 February 2011. It was conducted in two phases: Phase 1 – Basics of Molecular Biology and Bioinformatics (six days) and Phase 2 – Genomics and Proteomics (three days). Eighteen participants from different centers have undergone this training.

COMMENTS AND SUGGESTIONS OF THE REVIEW TEAM

PhytoFuRa is one of the commendable research initiatives of Indian Council of Agricultural Research, New Delhi to deal with *Phytophthora*, *Fusarium* and *Ralstonia* - the three major wilt pathogens affecting of horticultural and field crops. This project was in operation as an outreach programme of ICAR in the last 3 years of XI Plan.

Progress made by various institutes (IISR Calicut, CPRI Shimla, CPCRI Kasaragod, IIHR, Bangalore, CTCRI Trivandrum, NRC Citrus Nagpur, ICAR RC NEH Umaim, YSPUHF Kullu, IARI New Delhi, IIVR Varanasi, IIPR Kanpur, DOR Hyderabad, NRC Banana Trichy, CISH Lucknow, ICAR RC Goa, NBAII Bangalore and NBAIM Mau on various horticultural (*Apple, Banana, Black pepper, Chilli, Citrus, Coconut, Cocoa, Colocasia, Ginger, Guava Potato and Tomato*) and field (*Chickpea, Pigeon pea, Safflower*) crops were reviewed in the following aspects:

- Diversity study of pathogens *viz. Phytophthora*, *Fusarium* and *Ralstonia*
- Development of diagnostic and detection methodology
- Host-pathogen and microbe interaction studies
- Identification of host resistance using molecular tools
- Development of disease management strategies including IDM and biocontrol agents
- Development of Genomics & Bioinformatics supporting system

It was clear from the presentations made and reports submitted that significant advancements were made in collecting and assessing the pathogen diversity, their characterization and elucidation of pathotypes/races.

Good progress was made in developing diagnostics for species identification and detection from soil and host tissues.

Many new sources of host resistance were identified; molecular maps for tagging AVR genes were made in potato.

Transcriptome analysis of Black Pepper and Potato could reveal a better understanding of host-pathogen interactions, isolation of transcriptional factors and pathogen related genes.

Many promising isolates of *Trichoderma*, Actinomycetes and fungal endophytes were identified and evaluated and IDM strategies were developed.

Whole genome of two native isolates of *Phytophthora* and *Ralstonia* were sequenced for the first time.

Many online Bioinformatics resources like databases, interactive web tools, expert systems were developed for sequence archiving, literature survey, research progress reporting and monitoring (Table). Bioinformatics support has helped in sequence based pathogen identification, comparative genomics and diversity analysis.

Online resources and tools developed under PhytoFuRa

Sl.No.	Name of the resource/tool with URL	Description
1.	Phytoweb (www.phytofura.net.in/phytoweb)	Comprehensive database on *Phytophthora* species of horticultural/field crops; *Phytophthora* cultures maintained at IISR repository
2.	Phytophthora Genome Database (http://220.227.138.212/genomedb)	A database of whole genome sequence of black pepper isolate of *Phytophthora*
3.	PhytoPD (www.phytofura.net.in/phytopd)	A database of primer sequences related to various *Phytophthora* species
4.	Phytolib (www.phytofura.net.in/phytolib)	A bibliographic database on *Phytophthora/ Fusarium/Ralstonia*
5.	PhytoFuRa portal (www.phytofura.net.in)	A web portal for online monitoring of PhytoFuRa Outreach Project

The research outcome of the project has been published in various peer reviewed publications and research forums.

Research papers published	-	16
Research papers communicated	-	8
Research papers presented in Seminars/ Symposia	-	67

The progress made need to be consolidated further and translated into technology modules for the end users. It is suggested that these technologies need to be popularized and transferred to stake holders in aggressive PPP mode.

In view of the progress made and leads obtained the project need to be extended into the 12^{th} plan with the following focuses.

1. All the collections of pathogens and bio control organisms should be deposited at NBAIM, Mau and IISR, Calicut *(Phytophthora* and *Ralstonia)*
2. The leads obtained in detection of pathogens from soil, plant and seed samples need to be developed as cost efficient diagnostics kits.
3. The data generated in the host resistance and transcriptome sequencing need to validated and to be involved in convergent breeding programmes through gene stacking.
4. The whole genome data generated need to be annotated for better understanding of genome architecture. This may be extended to few more isolates and their host plants for data consolidation.
5. The leads obtained in disease management need to be converged as IDM modules and end products by keeping the 'farmer first' approach

Annexure 1

LIST OF PUBLICATIONS IN PHYTOFURA

RESEARCH PAPERS

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Annexure 2

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