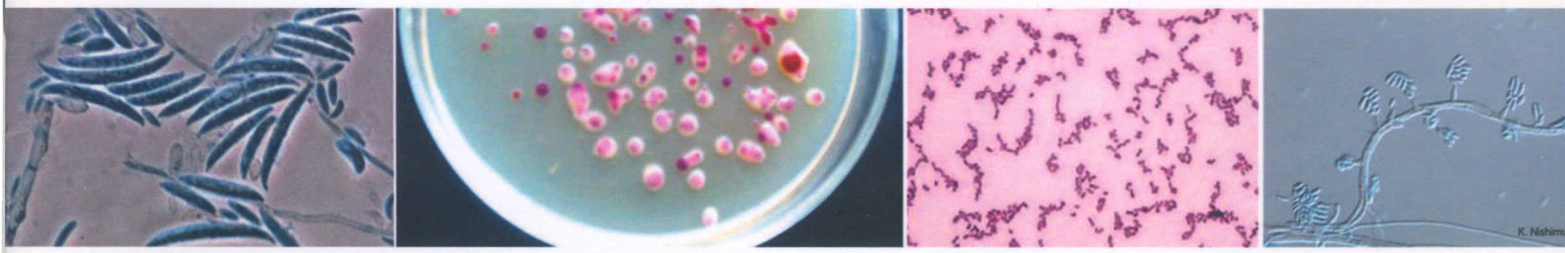
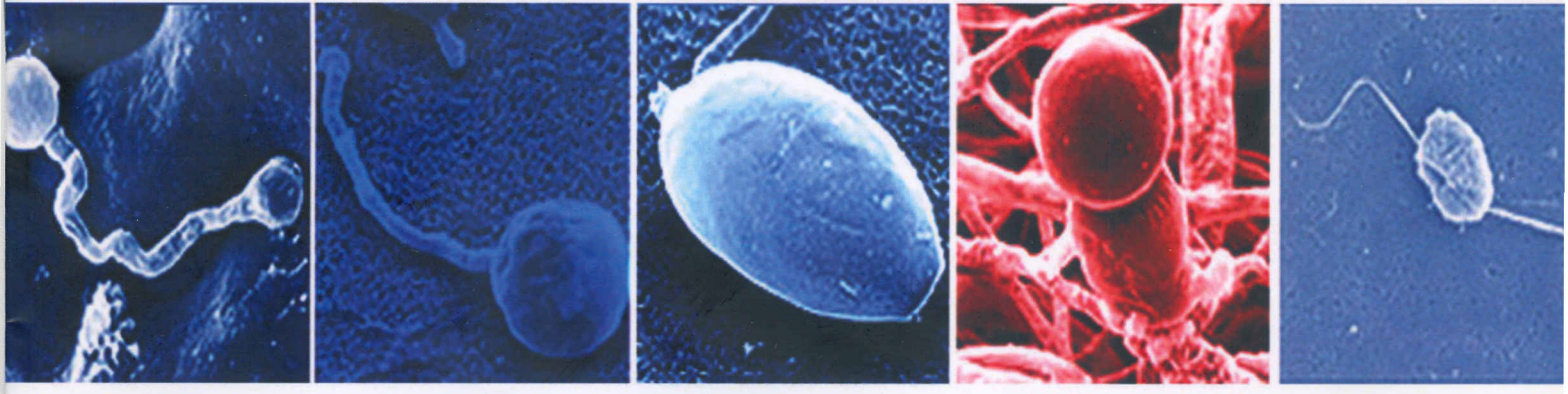
Outreach Project on *Phytophthora*, *Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops



PhytoFuRa
AN ICAR OUTREACH PROJECT ON
Phytophthora, Fusarium & Ralstonia

Salient Achievements



भारतीय मसाला फसल अनुसंधान संस्थान
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Contents

INTRODUCTION

PhytoFuRa is a major research initiative of Indian Council of Agricultural Research, New Delhi to comprehensively deal with the three major wilt pathogens *viz. Phytophthora, Fusarium* and *Ralstonia* affecting horticultural and field crops in a networking mode, launched on 23 February 2009 by Dr. H.P. Singh, DDG (Hort.).

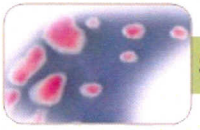
Organisms, Crops and Institutions involved

SUB PROJECT: *PHYTOPHTHORA*

- IISR, CALICUT
- CTCRI, TRIVANDRUM
- CPRI, SHIMLA
- YSPUHF RC, KULLU
- NRC CITRUS, NAGPUR & ICAR RC NEH, Umiam
- NBAII, BANGALORE
- CPCRI, KASARAGOD
- NBAIM, MAU

SUB PROJECT: *FUSARIUM*

- IIPR, KANPUR IARI, NEW DELHI
- NRCB, TRICHI
- DOR, HYDERABAD
- IIVR, VARANASI
- CISH, LUCKNOW
- PDBC, BANGALORE
- NBAIM, MAU



SUB PROJECT: *RALSTONIA*

- IISR, CALICUT NBAII, BANGALORE
- IIHR, BANGALORE IARI, NEW DELHI ICAR RC, GOA ICAR RC NEH, UMAIM NBAII, BANGALORE

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 MspI, AluI and RsaI) 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.

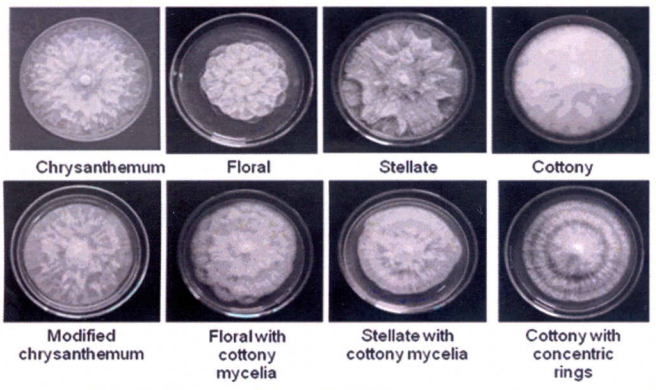
- *P. insolita* (NRCPh- 119) was isolated from water accumulated under the canopy of a Nagpur mandarin tree from Nagpur region for the first time from India.
- Molecular characterization of *Phytophthora* isolates from coconut and cocoa confirmed the association of *P. nicotianae* and *P. capsici* with 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

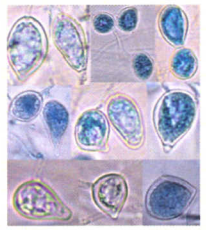
Sl. No	Host plant	No. of isolates	Sl. No.	Host plant	No. of isolates
1	Black pepper	186	20	Piper chaba	04
2	Colocasiae	60	21	Tapioca	03
3	Tomato	09	22	Bauhinia	02
4	Vanilla	03	23	Potato	302
5	Coconut	138	24	Papaya	01
6	Strawberry	03	25	Clove	01
7	Crossandra	02	26	Carnation	01
8	Gerbera	02	27	Vigna	02
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**

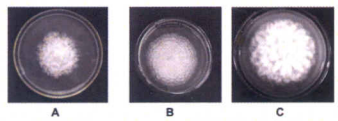
- The AFLP analysis of 15 *P. colocasiae* isolates from taro revealed high level of genetic diversity and grouped the isolates into three major clusters.
- Sequence based haplotype and population genetic studies indicated high variability in black pepper burrowing nematode populations compared to global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



Colony and sporangial morphology of black pepper isolates

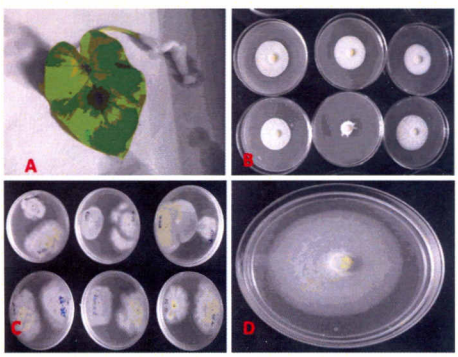


Sporangial variations of the different isolates of *Phytophthora* from Northeast India

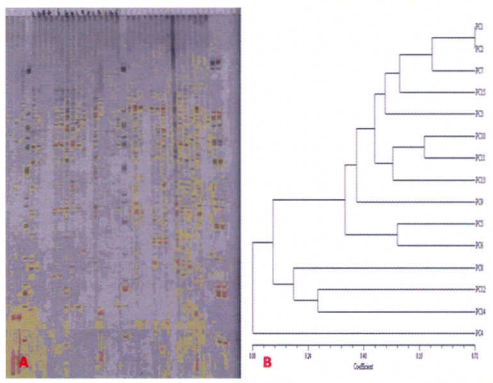


Citrus isolates A. *P. nicotianae* B. *P. palmivora* C. *P. citrophthora*

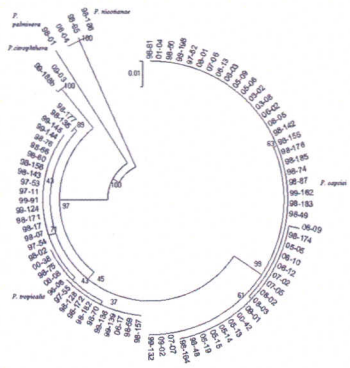
Virulence and molecular diversity



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

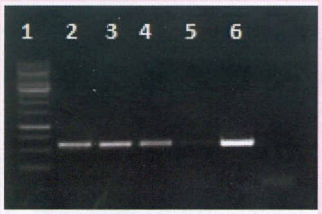


A: Urea-PAGE gel, B: Dendrogram depicting the genetic relatedness of *P. colocasiae* by AFLP analysis

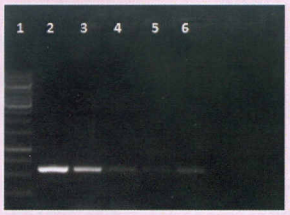


Dendrogram depicting high diversity among 126 *Phytophthora* isolates from black pepper

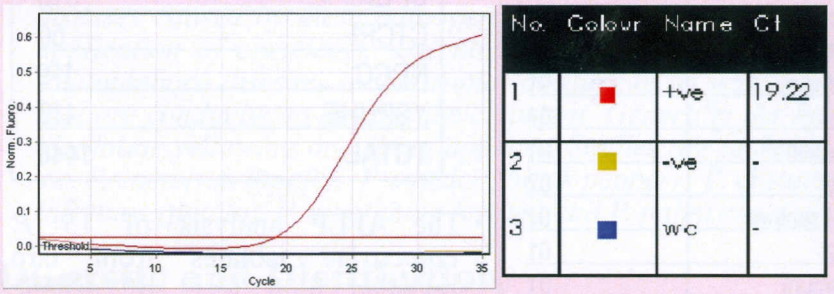
Diagnostics



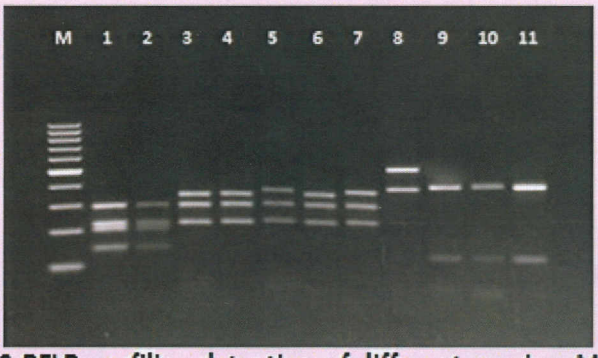
Detection of *P. infestans* from diseased tissue.
1=Ladder, 2= Symptomatic tissue, Healthy tissue (3= 5 mm away, 4= 10 mm away, 5= 20mm away), 6= DNA of *P. infestans*, 7= Control (un-inoculated tuber)



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

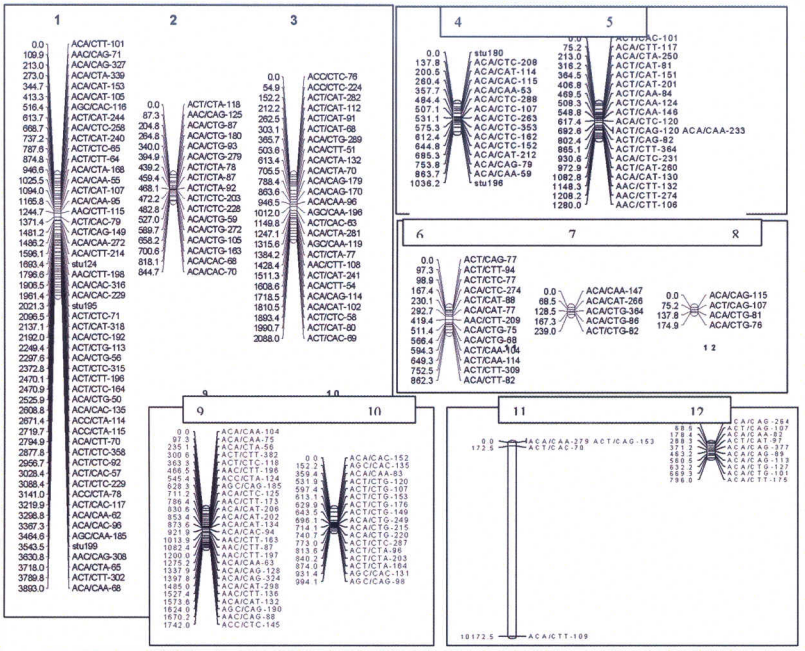
Host Resistance

Resistant / tolerant lines identified

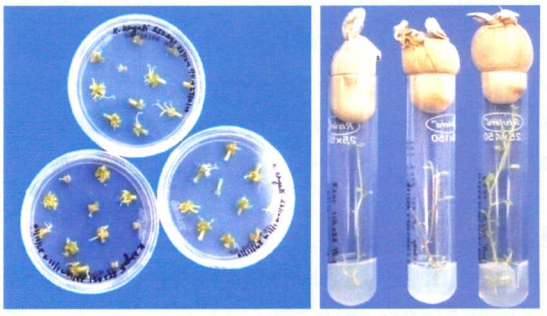
Crop	Promising lines
Apple	M9, M26, *Malus prunifolia* and *Malus floribunda* (rootstocks) and Vance Delicious (cultivar) highly resistant against *P. cactorum*
Black pepper	Acc. No. 1324 (Aimpiriyan) and H P 780 (Perambramundi X Karimunda)
Citrus	Rough lemon x Trifoliate hybrid and Troyer citrange (Chethali) 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.

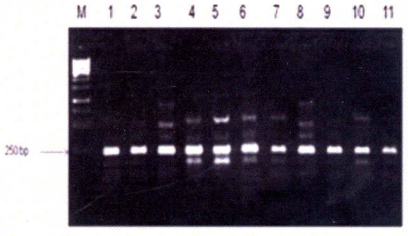


Molecular linkage map of S. chacoense



Potato transformation with Avr3a RNAi gene constructs iIR-Avr3a.

- *Phytophthora* Avr3a 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.
- 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.
- 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.



Expected Size 220bp
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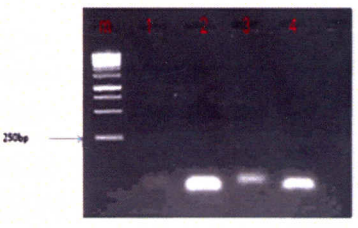
TCATGTGCGCGTCGACGGCGTGCAACACGATGATCTCCAAGATCGTGTCGCTGAACCC
CCTGACTGCGAGCTGACGGTGCCCACGAGCGGTCTGGTGCTCAACGTTAACTCGTACGCGAACGG
GTTCTCGACGAGTCCGAGAGGATCCTACTGTTCCGAGATGAGCTACATGGTTCGACGGC
CAACGGGGCCCACATTTCCTTGATTCAGAGCAGATCGTGGGCAAGTTGAAAAAATGCCCAAAAGTT
TTAAAA

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



Expected Size 143 bp
CGATTCGAGAGTTGATAGACTACGTTGCTTATTATCCTCCTGAGTATGACCCCGAGACAGTGACACCTT
GCCAACATACGAGTACTGCTCCTCCTGGAGTAAAACTGAGCTTCCACGTCCTGCAG
TGCTGAACTTCCATCTTCGGTCATGAACCTCGCCGGATCGACTACCCCGCGAGTCACCCGCTGTCCAA
AATAGGTTGTTACAACAAAGAGCCTGTTGCTGGAGAAAAAATCCACATATTTGTAAT
TAACGCATCCCTTCGACATTTTTAACGAAGGAACTGTTACTCACTTGTTAACTTCCTTGTGGGTAACGT
ATCCGGGTTCAAAGCTCTGTTGCTCCACGTTTGGAAGATTTGCGAATCCCTGTTG
TATGAAAAACTTCCCAGGACCGCCTCACGGTATCCATGTCGAGAGAGAAAATTTACACATGTATGCTCC
CCTCTATTGAGAAGCATAAAAAACCTAAATTGGCGTTGTCTGCCAAAACTACCGAC

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

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.

Epidemiology

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
NBAII Bangalore		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.

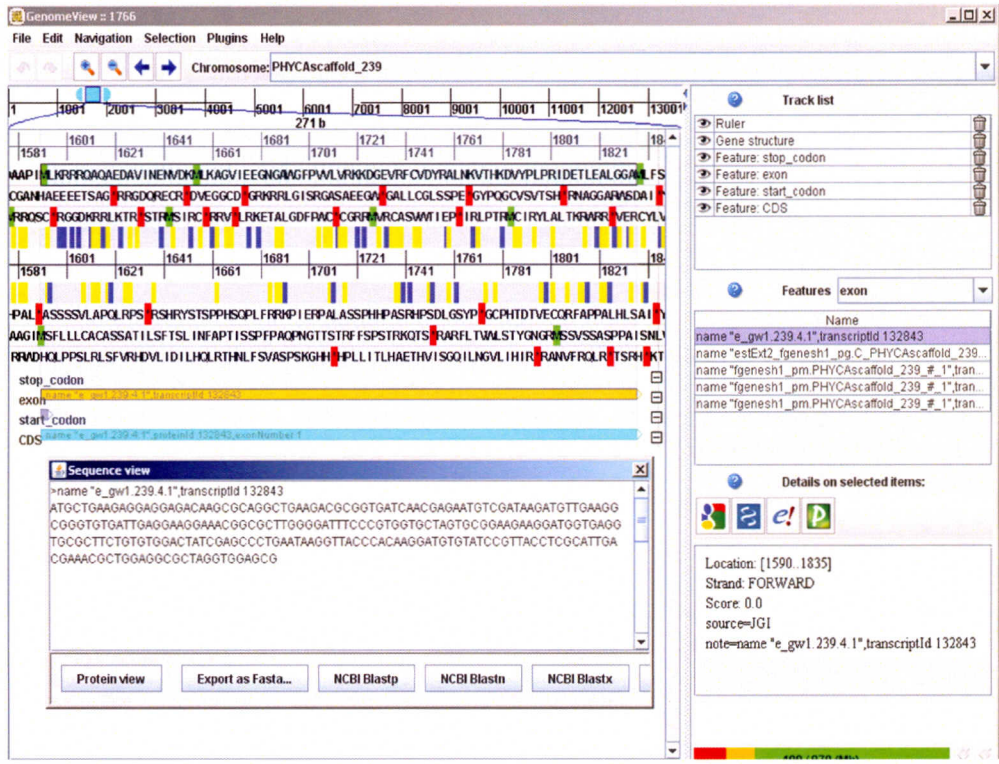
Genomics and Bioinformatics

WHOLE GENOME SEQUENCING

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*

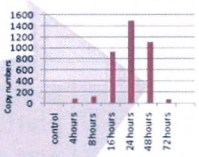
Organism	Number of scaffolds	Nucleotide composition				Genome Size (Mb)	Number of genes	Percentage Identity with others
		A	C	G	T			
P. capsici (IISR)	917	23.04%	26.43%	27.13%	23.40%	64.05	19,805	
P. capsici (JGI)	917	23.04%	26.43%	27.13%	23.40%	64	19,805	40%
P. infestans	4921	25.11%	23.39%	26.10%	25.40%	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 gene 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.

Transcriptome analysis of *Piper colubrinum*



Expression studies of osmotin gene using Real time PCR showed a gradual increase in copy no. of the gene in 24 h and a further reduction in copy no. Similarly the expression studies for β- 1, 3 glucanase gene showed a gradual increase in copy no. of the gene up to 48h and a further reduction

Genes identified in the transcriptome of *Piper colubrinum* challenged with *Phytophthora*

Stress inducible genes	Genes involved in Biosynthesis of secondary metabolites
Betaine aldehyde dehydrogenase	CHI chalcone isomerase
catalase	Chalcone synthase
Chitinase class I & VII	cinnamate 4-hydroxylase
glutathione-S-transferase	cinnamoyl-CoA reductase
Peroxidase	geranyl geranyl pyrophosphate synthase
Beta 1,3-glucanase	hmg-CoA reductase
Cu/Zn superoxide dismutase	lycopene beta cyclase
manganese superoxide dismutase	phenylalanine ammonia lyase
MAP kinase	p-coumaroyl shikimate 3'- hydroxylase
Osmotin	Transaldolase

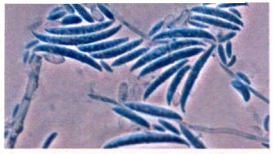
Summary statistics	*Piper colubrinum*	*Piper nigrum*
Sequence File Size	37.70 MB	76.06 MB
Maximum Sequence Length	15769	10479
Minimum Sequence Length	100	100
Average Sequence Length	567.844	721.922
No. of Sequences	62619	101284
Total Sequences Length	35557875	73119148
Total Number of Non-ATGC Characters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

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 and Distribution

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 NRC Banana	Chickpea Pigeon pea Banana	*F.oxysporum* f.sp. *ciceri,* *F. udum,* *F. oxysporum* f.sp. *cubense*	70 40 180

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

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

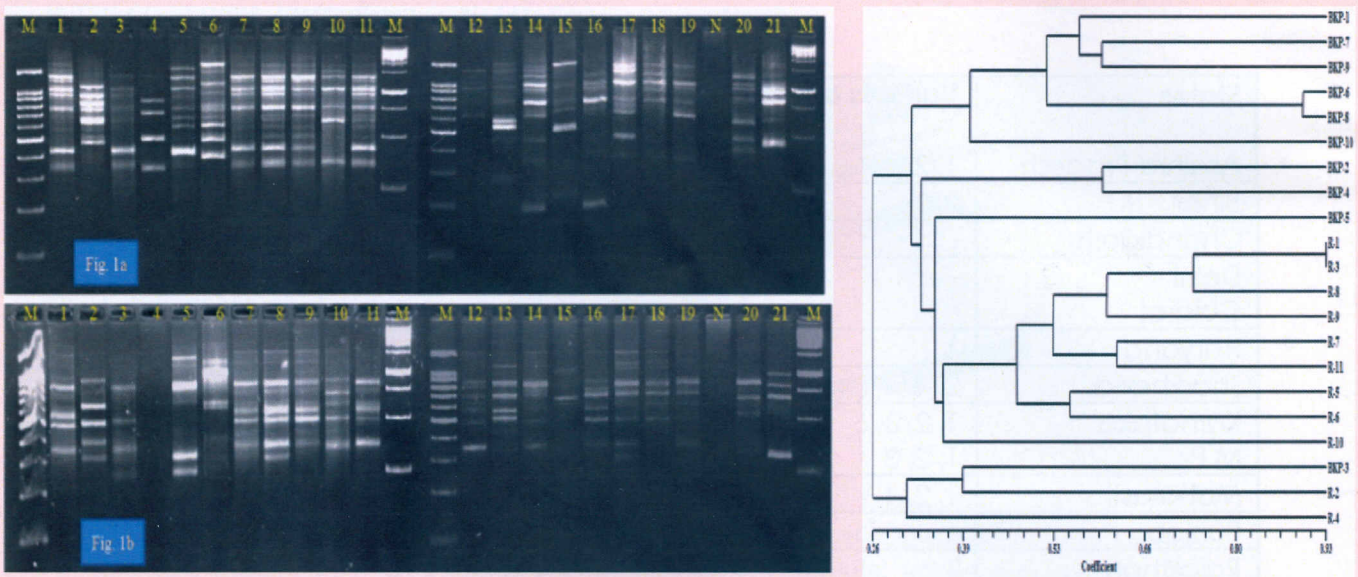
Morphological charecterisation

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*)

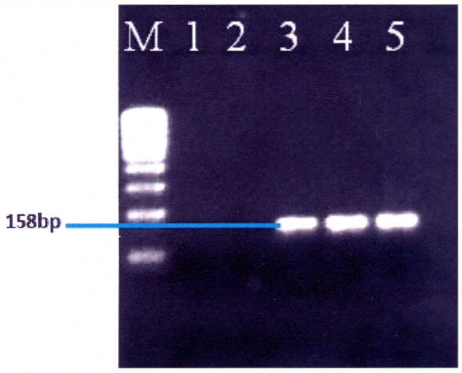


RAPD profiling (Fig. 1a&1b) and dendrogram of 21 *F. oxysporum* f. sp. *psidii* isolates derived from common data of RAPD fingerprints generated by UPGMA

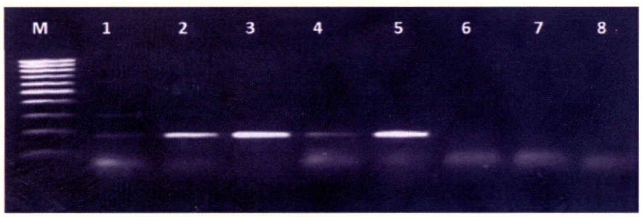
Host resistance

Crop	Promising lines
Safflower	Hybrids of *C. tinctorius* x *C. glaucus*, *C. tinctorius*x *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, PI397430, 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**



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 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.*

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

Disease management

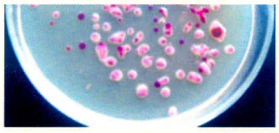
Promising biocontrol agents

Disease	Bio control agent
Fusarium wilt of pigeon pea	*Trichoderma viride* (Kanpur)
Fusarium wilt of chickpea	*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



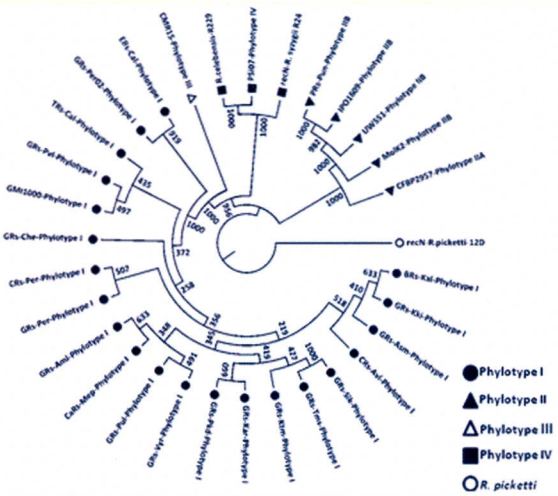
RALSTONIA

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 and Distribution

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.

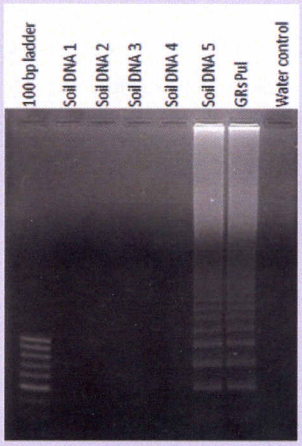
Institute	No of isolates	Crops
IISR, Kozhikode	30	Ginger, Small Cardamom
IIHR, Bangalore	174	Tomato,,Pepper,,eggplant
IARI, New Delhi	146	Tomato,,Capsicum,,Chilli,,Eggplant,,Potato
ICAR RC Goa	233	Tomato,,Capsicum,,Chilli,,Eggplant,,Marigold,,Sunflower



- 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.

Diagnostics



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

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.

- *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.

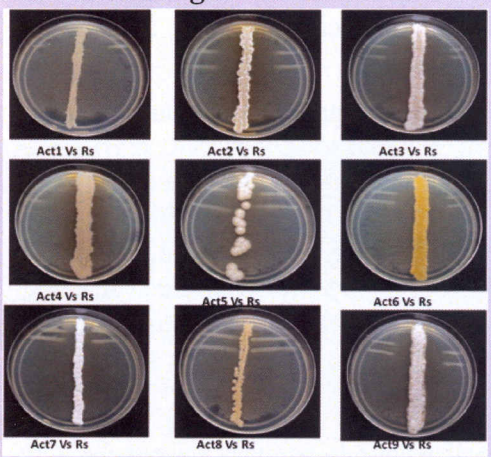
Host resistance

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 brinjal 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.

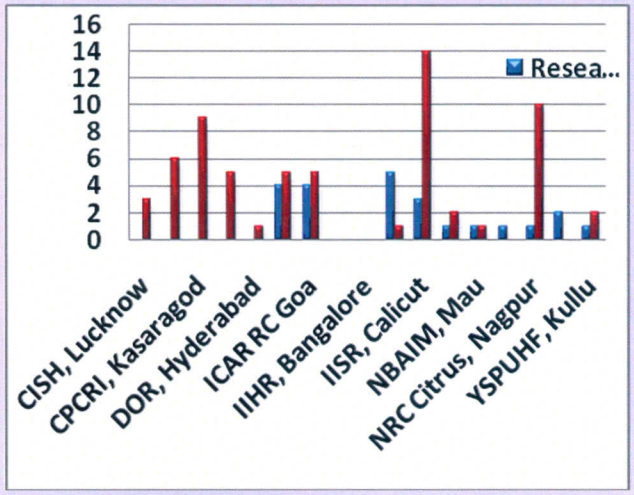
Infrastructure Facilities Built

Central Molecular Biology Facility:		IISR
Real time PCR	:	IARI, IISR, NRCC, NRCB
Tangential flow filtration unit for purification and mass production of bacteriophages	:	NBAII
2D gel electrophoresis	:	NRCB

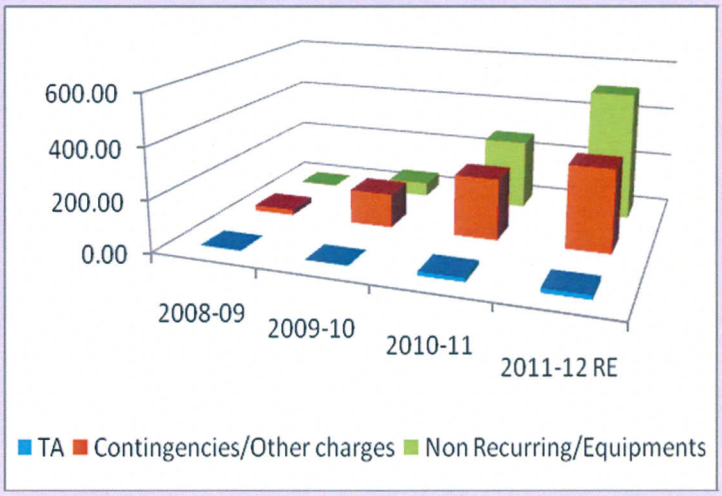
HRD

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.

Publications



Budget



Information storage, retrieval and project monitoring

Developed web based tools for information storage, retrieval, protocols, reporting and monitoring of project progress

Future Vision – 12 plan

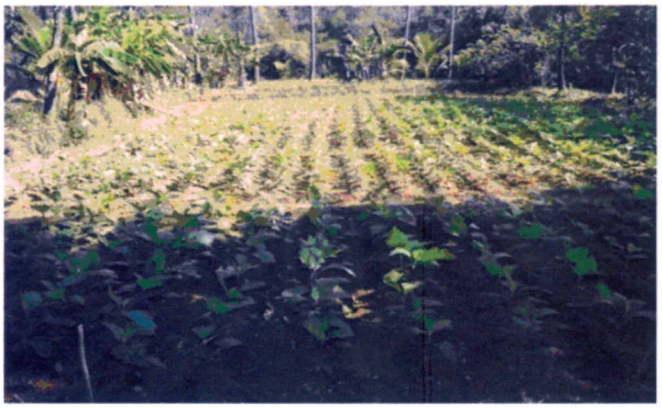
1. Completion of characterization, conservation and development of user friendly databases depicting the diversity of pathogens, biocontrol agents and their hosts.
2. Development of detection kits for field and other samples
3. Studies on allelic variations among members of resistance genes and other candidate genes and their expression (Targeted resequencing & allele mining).
4. Identification of resistance sources, tagging, convergent breeding for multiple resistance and MAS
5. Whole genome annotations, SNP development and gene isolation
6. Whole genome sequencing of hosts- Black pepper, Potato, Coconut etc
7. Development of IDM technology and GAPs for disease management and high productivity.
8. Development of transgenic/ cisgenics systems for crop improvement
9. Publication of information generated so far in to a reference book.

Management of bacterial wilt in brinjal using biocontrol agents
Field trial - Rabi 2011

ICAR Field

Taleigao Field

Pilar Field-1



Pilar Field-2

Ela Farm Field

Sulabhat Field

PhytoFuRa
AN ICAR OUTREACH PROJECT ON
Phytophthora, Fusarium & Ralstonia

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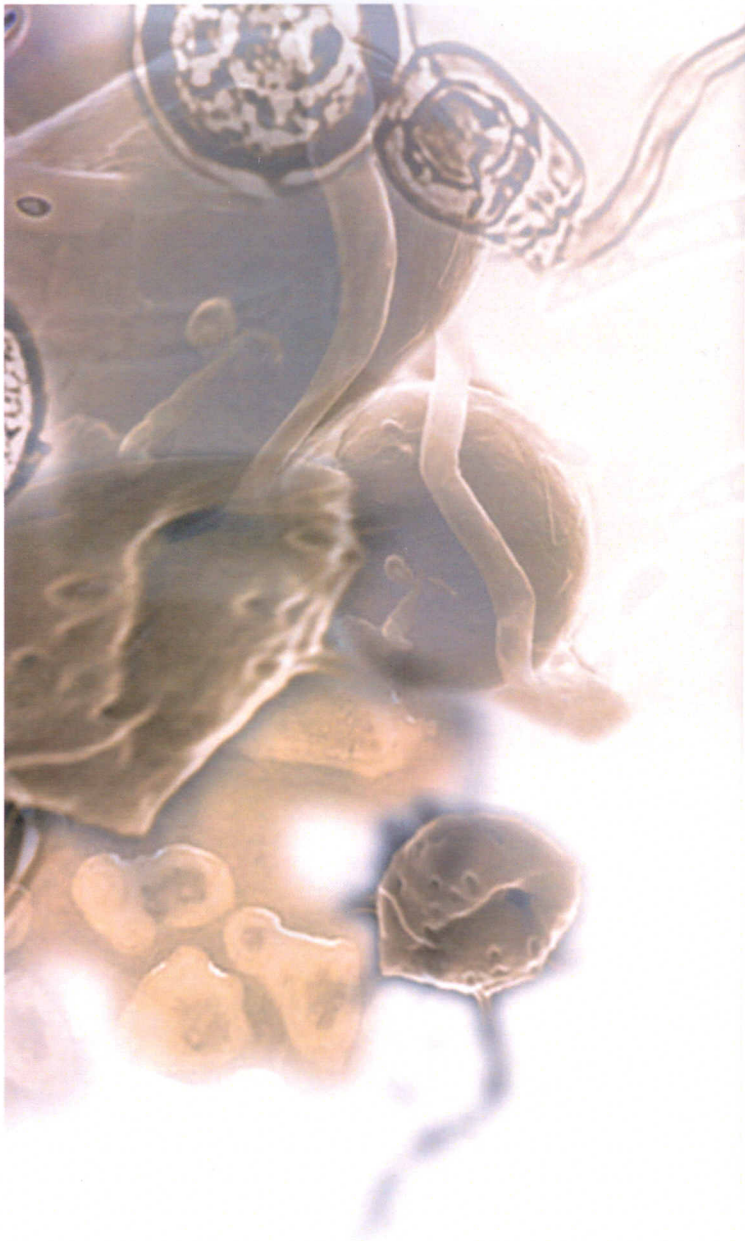

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