PhytoFuRa

AN ICAR NETWORK PROJECT ON
Phytophthora, Fusarium & Ralstonia

Outreach Project on *PHYTOPHTHORA, FUSARIUM* AND *RALSTONIA* DISEASES OF HORTICULTURAL AND FIELD CROPS

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PREFACE

Wilt pathogens have an enormous impact on the national economy by destroying valuable crops. Among these, the role of oomycete pathogen *Phytophthora* spp., fungal pathogen *Fusarium* spp. and bacterial wilt pathogen *Ralstonia solanacearum* are quite significant. They cause havocs to a number of horticulture and field crops of the country. Better understanding of fungal-plant interactions and pathogenicity factors is a crucial prerequisite for the development of novel disease control strategies. Recent advances in sequencing and genomic techniques have made it possible to monitor gene expression changes at the whole-genome level, which has impacted all aspects of biological sciences. Because our knowledge of molecular mechanisms of fungal pathogenesis is limited, comparative and functional genomic studies offer great promise to improve our understanding of host-pathogen interactions.

Many important plant pathogenic fungi like the ascomycetes *Fusarium graminearum,* the causal agent of Fusarium head blight (FHB) of wheat and barley and *F. verticillioides*, a causal agent of kernel and ear rot of maize, three *Phytophthora* species viz. *P. infestans*, *P. sojae* and *P. ramorum* as well as *R. solanacearum* have been completely sequenced. Comparative genomics is a powerful approach to address evolutionary and phylogenetic questions. In closely related plant pathogenic fungi/bacteria, comparative analysis can be used to improve de novo gene prediction and identify genes involved in host range determination, infection-related morphogenesis, and virulence.

One challenging task for the research community will be to apply the rich information gained in genomic studies to improve crop production and agricultural practices. As genomics and proteomics studies are not cost-effective, the research community has to combine resources by promoting collaborations within the community as well as between industry and academia. To facilitate and encourage this, Indian Council of Agricultural Research (ICAR) has launched an Outreach Project on *Phytophthora*, *Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops in the year 2008. The project envisages to study the diversity of these pathogens and host-pathogen-microbial interactions, aims to develop diagnostic and detection methodology for each of them and to develop management strategies against them. The project is operational in 17 centres distributed in nine states. Infrastructure build up and human resource development are also planned through this project. The six thematic areas are: Biodiversity, diagnostics, epidemiology, genomics, host resistance, disease management and HRD.

This publication details the research progress achievements made during the first year of its operation (from April 2009 to March 2010). A summary of achievements is given at the beginning of each chapter followed by the salient achievements of each centre. I take this opportunity to thank all the investigators of the project for their commendable contribution. I would like to thank Dr. S. Ayyappan (Secretary, DARE and Director General, ICAR), Dr. Mangala Rai (former DG, ICAR) and Dr. H.P. Singh (DDG, Horticulture) for their valuable guidance and timely advice in shaping up this network project. Acknowledgments are also due to ICAR for funding the project.

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National Coordinator (PhytoFuRa) & Project Coordinator (AICRP Spices)

EXECUTIVE SUMMARY

PHYTOPHTHORA

Biodiversity studies

Collection and characterization

Occurrence and distribution of major *Phytophthora* diseases of various crops were recorded from different locations. Altogether a total of 1517 samples were collected from different crops cultivated in various states of the country. From these 807 *Phytophthora* isolates were obtained. Among the disease endemic areas, the highest incidence of bud rot of coconut was observed in Kozhikode District of Kerala closely followed by Udupi District of Karnataka. Black pod disease of cocoa was found occurring in 84.3% of the gardens surveyed. The highest incidence of stem canker was observed in Andhra Pradesh followed by Tamil Nadu. Severe decline and death of Nagpur mandarin plants was observed in Vidarbha region (Nagpur and Amravati districts) of Maharashtra, Chhindwara District of M. P. and Hosiarpur region of Punjab. Maximum incidence of collar rot disease was observed in apple orchards at Karjan (Kullu District), Chuini -Ruhanda (Mandi District), Pujarli, Tikkar and Thanedhar (Shimla District) and Lippa (Kinnaur District) of Himachal Pradesh. Surveys were carried out in black pepper gardens of Kerala and Karnataka to collect new isolates of *Phytophthora* and plant parasitic nematodes.

Isolation of Phytophthora

Phytophthora was isolated from infected samples by direct plating in selective media and through baiting technique. Incidence of *Phytophthora* was significantly high in samples collected from coconut mixed cropping systems than monocropping system. Similarly the incidence black pod and stem canker diseases was more when cocoa was mixed cropped with either coconut or arecanut. All the surveyed citrus orchards were found to be infested with *Phytophthora* spp. viz. *P. nicotianae* and *P. palmivora.*

Phytophthora repository

The repository of *Phytophthora* at Indian Institute of Spices Research has been revived with about 241 isolates from different horticulture crops like black pepper, cardamom, betel vine, colocasia, cocoa, cassava, tomato, potato, arecanut and coconut (Table 1). The new collections made during the year (43 Nos.) were added to the repository bringing the total collections to 284.

Table 1. *Phytophthora* isolates in the repository at IISR, Calicut

Sl. No.	Crop	No. of isolates		
		Collections	Collections made during 2009-10	Total
1	Pepper	126	35	161
2	Colocasia	09	-	09
3	Tomato	11	-	11
4	Vanilla	03	-	03
5	Coconut	07	1	08
6	Strawberry	03	-	03
7	Crossandra	02	-	02
8	Gerbera	01	01	02
9	Periwinkle	03	03	03
10	Betel vine	24	-	24
11	Cardamom	04	-	04
13	Cocoa	07	01	08
14	Rubber	08	-	08
15	Capsicum	02	-	02
16	Nutmeg	03	-	03
17	Citrus	10	01	11
18	Others	18	01	19
	TOTAL	**241**	**43**	**284**

Isolation of nematodes

The nematodes were extracted from soil by decantation followed by centrifugal flotation and from roots by direct examination through microscope. Altogether 82 samples were collected out of which 20 samples yielded *R. similis* while 11 samples yielded root knot nematode.

Characterization

The *Phytophthora* isolates collected are being characterized by studying their colony morphology, sporangial morphology, pathogenic variability, mating type determination, sensitivity to metalaxyl and also by employing various molecular tools. For taking up molecular studies, genomic DNA has been isolated from *Phytophthora* isolates standard protocols and various markers like RAPD, RFLP, SSRs and ITS sequencing were used (Table 2). DNA was isolated from plant parasitic nematodes *R. similis* and *Meloidogyne* too for assessing their diversity.

The optimum temperature and medium for culturing *P. colocasiae* and *P. cactorum* were identified.

Diagnostics

PCR detection using species-specific primer pairs was standardized for *P. nicotianae, P. capsici* and also *R. similis*. Attempts were also made to detect *Phytophthora* infection in citrus plants using PCR-RFLP method.

Table 2. Characterization of different isolates of *Phytophthora* by different PhytoFuRa centres during 2009-10

Species	Crop	Total isolates	Colony morphology	Sporangial morphology	Pathogenic variability	Mating type	Metalaxyl sensitivity	Molecular characterization			
								RAPD	PCR-RFLP	ITS sequencing	SSRs
P. cactorum	Apple	82	-	-	-	-	-	-	-	-	-
P. capsici	Black pepper	282	135	128	112	87	10	21	10	6	126
P. colocasiae	Taro	15	15	15	-	-	-	-	-	-	-
P. infestans	Potato	50	-	-	-	50	50	-	-	-	-
P. nicotianae	Citrus	64	16	16	-	16	-	9	32	14	-
P. palmivora	Coconut	136	56	35	35	-	-	-	-	-	-
	Cocoa	370	283	103	80	-	-	-	-	-	-
	Citrus	15	4	4	-	4	-	-	14	4	-

Epidemiology and host pathogen interaction

JHULCAST model, a model for assessing yield loss in potato was developed. Weather data is being collected for development of a Decision Support System.

Cell wall glucan elicitor of *P. colocasiae* has been isolated and its elicitation activity was assessed through bioassays and assay of â-1, 3- glucanase activity. Similarly, attempts were also made to isolate and characterize cell wall lipids of *P. colocasiae.* Host pathogen interaction studies were also initiated in citrus. Amplification of elicitin genes was obtained in *P. capsici* using specific primers designed from conserved sequences.

Genomics

A wound inducible promoter, PVS3, has been detected in a taro cultivar, Sree Kiran, by amplifying the genomic DNA with PVS3 specific primer.

Gene silencing

Genes carrying *RXLR* motif and CRN family of genes were mined from *P. infestans* genome. The *Avr3a* 14368 whole genome sequence was chosen for development of an inverted repeat RNAi gene construct.

Similarly, *P. infestans* genome was searched for the presence of *CRN* family of genes for RNAi targeting and the preliminary search revealed the presence of 23 *CRN* gene families. A number of potential amiRNA sequences were designed for artificial microRNA-mediated silencing of *P. infestans* avirulence gene, *Avr3a.*

Host resistance

Screening of rootstocks/germplasm

Out of the six citrus rootstocks screened against *P. nicotianae*, only one rootstock i.e. Rough lemon x

Trifoliate hybrid, was found moderately susceptible. Eleven apple rootstocks were screened against *P. cactorum* by exicised twig method and M9, M26, *Malus prunifolia* and M26, M9, *Malus prunifolia,* and *Malus floribunda*In were found resistant. Among the 20 different apple cultivars screened, cv. Vance Delicious was highly resistant while Royal Delicious was moderately resistant. In black pepper, 50 parental lines were screened for their reaction to *P. capsici* using leaf, stem and root inoculation techniques.

Identification of resistance genes

Studies have been initiated to locate and amplify R genes in potato lines using specific primers. For taking up DDRT-PCR studies, a protocol for isolation of black pepper RNA was standardized using acid–phenol guanidinium thiocyanate method. This method gave good yield of RNA with low amount of DNA contamination. Eight pairs of degenerate primers were designed from conserved P-loop and GLPL motifs of NBS-LRR regions of six *Phytophthora* resistance genes and amplification of R gene candidates is in progress in black pepper.

In potato, DNA was extracted from late blight differentials, 44 Indian potato varieties and 43 advanced hybrids and its qualitative and quantities analysis was done. Primers were synthesized for markers SPUD 237 and R1AS (R1gene) and cLET5E4 and GP 185 (R3a gene) and validation is in progress.

Two scar markers developed earlier for *Phytophthora* resistance in black pepper and in C*apsicum annuum* (Phy 5.2), respectively, are being tested and validated in selected black pepper lines and related species, reported to be resistant to *P. capsici*.

Association mapping and construction of linkage map

Two mapping populations (Panniyur 1 x Subhakara and P24 X Subhakara) were raised to study the inheritance of *Phytophthora* resistance in black pepper. Morphological and molecular profiling of these populations is in progress. Besides, a selfing (OP) programme was done to develop F3 progenies of Perambramundi - P24 - P24 O4. About 300 progenies of *P. colubrinum* segregating for *Phytophthora* resistance were developed for tagging and isolating the genes with differential display. ISSR profiling of 75 P1 x K1 progenies is in progress in order to construct a linkage map.

In potato, all the 126 F1 offspring of the cross *S. spegazzinii x S. chacoense* were maintained in vitro as micro plants. The population was genotyped using 4 AFLP primer combinations and 1 SSR primer set. A molecular map of *S. chacoense* developed using 134 markers that segregated as per the expected 1:1 ratio.

Disease Management

Non chemical management

Leaf water extracts of *Vitex negundo*, *Melia azadirachta*, tirmira (*Xanthoxylum esculantum*), mustard (*Brassica oleracea*), *Murraya koningii*, and castor were highly effective in inhibiting the growth of *P. cactorum*.

Soil solarisation studies have proved the eradication of *P. cactorum* in upper layers (0-30 cm) of infested soils.

Biological control

Attempts were made to isolate, identify and evaluate new fungal and bacterial antagonists of different *Phytophthora* isolates. Efforts of the past one year are summarized in Table 3.

Table 3. Isolation and evaluation of new fungal and bacterial antagonists against *Phytophthora*

Crop	Target species	Biocontrol agent	No. of isolates collected	In vitro bioassay	In vivo bioassay	Promising bioagent
Apple	*P. cactorum*	Antagonistic fungi	32	32	N S	-
		Rhizobacteria	23	23	N S	-
Black pepper	*P. capsici*	Antagonistic fungi	74	74	N S	-
		Endophytic fungi	80	70	N S	-
		Rhizobacteria	94	94	N S	-
Citrus	*P. nicotianae*	Antagonistic fungi	51	46	5	*T. harzianum* (NRCfBA 43 and 44)
		Rhizobacteria	19	19	N S	-
Potato	*P. infestans*	Rhizobacteria	25	25	N S	-

Two isolates of *Trichoderma harzianum* were tested for their biocontrol activity against *P.colocasiae* by dual culture technique. *Trichoderma* was mass multiplied using solid fermentation on neem cake and wheat bran in the ratio 1: 2. The spore count and shelf life of this formulation was also studied. In chillies, *T. harzianum* isolates having induced systemic resistance were short-listed by assessing their ability to induce phenol, peroxidase and glucanase. Cell wall elicitors were extracted from *T. harzianum* isolate Th10 and its efficacy was tested by studying their ability to induce the glucanase and peroxidase in chilli plants as well as to protect the chilli plants from *P. capsici* infection.

Eighty isolates of endophytic fungi were isolated from different cultivars and varieties of black pepper and the antagonistic potential of these isolates was evaluated through dual plate assay. Out of the 70 isolates tested for antagonistic properties, only 15 isolates were found to have antagonism against the test pathogen, *P. capsici.* Morphotyping and cultural characters of each isolate is being studied.

A field trial is in progress to evaluate the efficacy of three each endophytic bacteria and rhizobacteria for the management of *Phytophthora* and plant parasitic nematodes infesting black pepper. Another field trial has been laid out to evaluate three promising disease/ nematode resistant black pepper lines viz. Hp 39, IISR Shakti, C 1090 and biocontrol agents in combination with *T. harzianum, Pseudomonas fluorescens* (IISR 6 and ISR 853) and *Pochonia*.

To study the effect of temperature on efficacy of fungicide, four fungicide viz, Acrobat, Curzate (Cymoxanil + Mancozeb) Mancozeb, Ridomyl (Metalaxyl + Mancozeb) were tested at 25^{0}C.

Bioinformatics and IT support

EST assembly and annotation

ESTs (56,457 numbers) of *P. capsici* available from dbEST of NCBI were downloaded, cleaned and assembled into 5966 contigs using CAP 3 program. Functional annotation of these contigs was performed using BLASTx. The analysis has revealed that 84.73% of the ESTs displayed significant similarity to known sequences in GenBank. About 3.57% (213 numbers) of ESTs were assigned to hypothetical proteins of unknown function while 699 (11.7%) had "no hits." The analysis of ESTs enabled to identify a range of genes likely to be involved in pathogenesis, drug resistance, stress, host degradation and genetic marker related protein for *Phytophthora* identification.

Primer designing

About 223 microsatellites were detected in EST sequences of *P. capsici* using MISA. Primer pairs were designed for the identiûed SSRs using Primer3 software. Nucleotide sequences of elicitins from different *Phytophthora* species were downloaded from NCBI and compared using ClustalW to study the conserved motifs. Based on these conserved motifs six sets of primers were designed and are being evaluated in the wet lab. Eight pairs of degenerate primers were also designed for NBS-LRR resistance gene identification in *Piper colubrinum*.

PhytoWeb, a database on Phytophthora

PhytoWeb, a comprehensive portal on *Phytophthora* diseases of horticultural crops in India was developed by modifying the existing 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 IISR repository.

Portal for online monitoring of PhytoFura

A web interface for monitoring the PhytoFuRa project on a real time basis was developed and hosted (www.phytofura.net.in). All participating institutes can login to the system and can submit their periodic progress reports and financial statements etc. through this web tool. The project leaders and policy makers can view the compiled periodic progress reports, financial statements etc.

Phytolib, a literature database

Phytolib, an electronic database of research publications on *Phytophthora* has been developed and launched through the above portal. Full texts of all the original articles on *Phytophthora* published since 2000 are currently made available in this database.

FUSARIUM

Collection and characterization

Surveys were conducted in respective areas for crops like banana, chilli, chick pea, guava, pigeon pea, safflower, tomato etc. and collected *Fusarium* viz. *F. oxysporum* f. sp. *cubense* (*Foc*)., *F. udum, F. oxysporum* f.sp. *ciceri, F. oxysporum* f. sp. *ciceris , F. oxysporum* f. sp. *lycopersici, F. solani,.* etc. A total of 516 new *Fusarium* isolates (180 from banana, 62 from chilli and tomato, 145 from guava, 70 from chick pea, and 51 from safflower have been added to the repository of *Fusarium* in addition to the earlier collection of 1198 in respective places viz. CISH, Luck now (Guava), DOR, Rajendranagar (Safflower), IIPR, Kanpur (Pigeon pea and Chick pea), IARI, New Delhi (Chickpeas), NRCB, Thiruchirappally (Banana) and IIVR, Varanasi (tomato and chilli) (Table 4). Besides 25 non-pathogenic isolates of *Fusarium* isolates have been collected at PDBC Bangalore and studied their antagonistic potential against pathogenic *Fusarium* from tomato using eight different methods. Most of the isolates collected were deposited in the National Bureau of Agriculturally Important Microorganism (NBAIM) Mau, where the isolates were conserved in mineral oil and in glycerol at -80^{0}C for short term conservation and lyophilized for long term conservation. They also characterised the isolates using morphological and molecular tools.

Table 4. Collection, conservation and characterization of *Fusarium* isolates

Fusarium species	No. of isolates collected		Morphological characterization	Molecular characterization
	Old	New		
F. oxysporum	-	58	58	-
F. oxysporum f. sp. *carthami*	51	-	-	54
F. oxysporum f sp. *ciceris*	378	97	-	56
F. oxysporum f. sp. *cubense*	-	180	-	-
F. oxysporum f.sp. *lycopersici*	28	43	62	-
F. solani	-	84	84	-
F. udum	7‘6	10	-	24
Non-pathogenic *Fusarium*	-	25	25	7
Fusarium sp.	-	19	-	19
Total	1198	516	229	160

Fusarium isolates at various centres were characterized into morphological and pathogenic groups and variants were identified. The morphological variability was studied on the basis of pigmentation, growth pattern, colony colour, mycelial colour, shape and size of micro conidia and macro conidia etc. and considerable variations were observed in conidial size as well as growth and pigmentation. Fifty four isolates of *F. oxysporum* f. sp. *ciceris* were studied for pathogenic variability and the reaction among two differential hosts indicated existence of four races among the isolates.

Molecular characterization was carried out for different isolates of *Fusarium* by ISSR, RAPD and ITS sequence analysis. Twenty nine pairs of *Fusarium* specific primers with 18-28 bases have been synthesized. By amplifying and sequencing the ITS region the identity of seven non-pathogenic *Fusarium* isolates, short listed from 25 isolates, was confirmed as *F. merismoides, F. solani, F. oxysporum* and *F.moniliforme.* Diversity analysis of *Fusarium oxysporum* f. sp. *carthami* was carried out by analyzing EF1á sequences.

Diagnostics

Markers designed from the ITS sequences of *F. oxysporum* f sp. *ciceris* during earlier wilt network project was used for amplifying the genomic DNA and the marker produced an amplification product of 292bp specific to the *F. oxysporum* f. sp. *ciceris.*

Epidemiology

Genomics

In banana, three RGAs primers namely S2/AS1, LRR F/R and NBS/LRR have been designed and amplified 500 bp, 400 bp and 850 bp, respectively in cv. Rose. The amplified products were cloned into pGMT vector and the sequencing of these fragments indicated that more than 90% of clones obtained showed homology to NBS domain of known R genes or RGAs from other species published in GenBank.

Host Resistance

Evaluation of genotypes of pigeon pea and chickpea in sick plots yielded 17 pigeon pea and 18 chickpea wilt resistant donors and are undergoing screening for resistance against variant 2, 4 and 5 of *F. udum* and races 1, 2, 3, 4, 5 and 6 of *Foc.* Multiplication of two banana accessions viz. cv. Rose and Nendran have been done *in vitro* using the shoot tip as explants.

Disease management

Biological control has been given priority for managing *Fusarium* diseases and for this, both rhizospheric and endophytic antagonistic organisms that inhibit spore germination and mycelial growth by producing non-volatile and volatile metabolites like HCN, chitinase, IAA etc. were isolated. Altogether 185 endophytic bacterial isolates, 43 endophytic and 44 rhizospheric *Trichoderma* isolates, 31 non-pathogenic *Fusarium* and 30 endophytic actinimycetes were evaluated against species of *Fusarium* besides thirty-three medicinal plant extracts. Out of the 185 endophytic bacterial isolates from 23 banana accessions resistant to *Fusarium* wilt disease eight promising bacterial isolates having multiple actions for pathogen suppression were short-listed.

Twelve isolates of endophytic *Trichoderma* sp. having multiple actions against *Foc* including phosphate solubilization were identified from 43 isolates collected from 13 different *Foc* resistant banana germplasm. Among the 19 isolates of *Trichoderma* spp. maintained at NRCB, one *T. harzianum* isolate showed maximum mycelial inhibition and 100% inhibition of spore germination. Quantification of phosphate solubilization by 29 effective isolates of *Trichoderma* spp. of rhizospheric and endophytic nature identified four isolates with high phosphate solubilization activity.

Application of *Actinomycetes* + *T. viride* K2T5 + *T. harzianum* was effective in controlling *Foc* with an internal score of 1.8 as against *Foc* score of 4.1 in the control. Preliminary observations indicated that *T. viride* strain from Dholi (Bihar) at 10 g/kg seed as most efficient in suppressing *Fusarium* wilt and enhancing the growth and nodulation in pigeon pea.

PGPR strains *Pseudomonas fluorescens* (Pf-80) and *Bacillus* species (Km-5) were found effective against *F. oxysporum* f. sp. *ciceris* by causing highest inhibition of mycelial growth. *P. fluorescens* (Pf-80) was found to be compatible with *T. harzianum* and *Rhizobium ciceri* and therefore, it could be selected for further integration.

Carboxin, thiophanate-methyl, tetramethyl thiuram disulphide, metalaxyl + mancozeb, captan and mancozeb inhibitory to *F. oxysporum* f. sp. *ciceris* were proved to be compatible with *T. harzianum*.

RALSTONIA

Collection and characterization

In India bacterial wilt is one of the important production constraints in crops such as tomato, brinjal, chilli, potato, and ginger in most of the states. During the period 2009-2010, a collection of 443 isolates of *Ralstonia solanacearum* representing diverse crops species such as tomato, chilli, eggplant, marigold, ginger and potato were made. The bacterial collection represented various Indian states such as Kerala, Goa, Karnataka, Tamil Nadu, Delhi, Orissa, Sikkim, Uttaranchal, Meghalaya, Andaman and Nicobar Islands, Himachal Pradesh, Uttarakhand, Jammu and Kashmir, West Bengal, Jharkhand .

Characterization

These isolates were characterized for various phenotypes such as pathogenicity on their respective hosts, and biovar. The results indicated the dominance of biovar 3 of *R. solanacearum* in India which confirms the global distribution pattern of *R. solanacearum*. Molecular methods for diversity analysis were standardized based on conserved gene sequences (16s rDNA, egl gene) and intergenic sequences (ERIC) in *R. solanacearum*. The results indicated that the bacterium displayed clear genomic diversity among the locations and crop origin. Full genome sequences from *R. solanacearum* GMI1000 was used to design primers for diversity analysis based on sequences of highly conserved DNA repair protein (RecN).

Diagnostics

Methods have also been standardized for rapid detection of the bacterial pathogen in plant tissues and soil, based on specific nucleotide sequences. The detection threshold is also determined to be 100 cells per gram of soil. However, biovar specific PCR based rapid detection is presently lacking.

Management

Significant achievements have been made in biological control strategy for bacterial wilt. Microbial candidates found effective for biological control of *R. solanacearum* are *Stenotrophomonas maltophila* (IISR, Calicut), *Bacillus subtilis* (IARI, New Delhi, ICAR-Complex, Goa), *Pseudomonas putida* (ICAR-Complex, Goa). Bacteriophage which lyses the *Ralstonia* cells were isolated from soils collected from various solanaceous vegetables (PDBC, Bangalore) which would pave way for a field control of bacterial wilt in future.

Of the 33 medicinal plant extracts screened, five plant extracts recorded 100 percent inhibition of spore germination and more than 1 cm zone of inhibition in agar well diffusion method.

PHYTOFURA: AN INTRODUCTION

Field and horticultural crops are attacked by a large group of plant pathogens inflicting moderate to heavy crop losses. Amongst these, diseases caused by *Phytophthora, Fusarium* and *Ralstonia* are most devastating. They affect large number of field and horticultural crops ranging from vegetables, fruits, spices, plantation crops, ornamental, pulses and oil seed crops. Major *Phytophthora* diseases are late blight of potato, citrus decline, black pod of cocoa, foot rot of black pepper, koleroga of arecanut *etc*. Chickpea, pigeon pea, lentil, castor, safflower, guava, banana, tomato, chilli, cucurbits, cumin, coriander and gladiolus are often attacked by *Fusarium* spp. causing crop losses ranging from 8 to 40 % in different parts of the country. Bacterial wilt caused by *Ralstonia solanacearum* in solanaceous and Zingiberaceae plants is destructive.

Most of the *Phytophthora* and wilt diseases are endemic in nature and therefore difficult to eradicate/ manage. A very high degree of pathogenic variability exists in all *Phytophthora* and wilt causing pathogens that makes the disease management more complicated. Non- availability of host resistance in most of the crops against these diseases has further aggravated the situation. The pathogens are seed borne and are often carried incipiently which necessitate development of sensitive and rapid detection techniques. Although there is lot of similarity amongst the *Phytophthora, Fusarium* and *Ralstonia* diseases yet, all the three group of diseases are class apart based on their ecology and biology and therefore need to be addressed individually.

Accordingly, Indian Council of Agricultural Research, New Delhi sanctioned and launched a new research initiative titled 'Outreach Project on *Phytophthora*, *Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops (PhytoFuRa). PhytoFuRa is envisaged as a network project with three separate sub projects on a) *Phytophthora*, b) *Fusarium* and c) *Ralstonia*. It was launched on 23 February 2009 by Dr. H.P. Singh, DDG (Hort.). The objectives of PhytoFuRa are as follows.

- To study the diversity of pathogens *Phytophthora, Fusarium* and *Ralstonia*
- To develop diagnostic and detection methodology for *Phytophthora, Fusarium* and *Ralstonia*
- To study the host-pathogen and microbes interaction using genomic tools
- To use molecular and genomic tools for identification of resistant types
- To develop management strategies including biocontrol agents for facilitating management of *Phytophthora, Fusarium* and *Ralstonia* diseases.

Table 5. Crops and corresponding institutes involved in the PhytoFuRa Outreach Project

Sub-project I: *Phytophthora*

Crop	Institutes
Black pepper	Indian Institute of Spices Research, Calicut
Potato	Central Potato Research Institute, Shimla
Citrus	National Research Centre for Citrus, Nagpur; ICAR Research Complex for NEH Region, Umiam
Coconut and cocoa	Central Plantation Crops Research Institute, Kasaragod
Colocasia	Central Tuber Crops Research Institute, Trivandrum
Apple	Dr. Y.S. Parmar University of Horticulture & Forestry, RC, Kullu

Sub-project 2: *Fusarium*

Crop	Institutes
Chickpea and pigeon pea	Indian Institute of Pulses Research, Kanpur; Indian Agricultural Research Institute, New Delhi
Safflower	Directorate of Oilseeds Research, Hyderabad
Guava	Central Institute of Sub-tropical Horticulture, Lucknow
Banana	National Research Centre for Banana, Trichy
Solanaceous vegetables (tomato, chilli)	Indian Institute of Vegetable Research, Varanasi; National Bureau of Agriculturally Important Insects, Bangalore

Sub-project 3: *Ralstonia*

Ginger	Indian Institute of Spices Research, Calicut; National Bureau of Agriculturally Important Insects, Bangalore
Solanaceous vegetables (Tomato Brinjal, Chilli)	Indian Institute of Horticultural Research, Bangalore; Indian Agricultural Research Institute, New Delhi; ICAR Research Complex for Goa; ICAR Research Complex for NEH Region, Umiam; National Bureau of Agriculturally Important Insects, Bangalore

SUBPROJECT - 1
PHYTOPHTHORA

PHYTOPHTHORA

Plant pathogenic oomycetes cause diseases on all types of plants, in all sorts of environments, around the world. There are over 83 species of *Phytophthora* that are particularly destructive. They exhibit morphological features analogous to pathogens in Kingdom Fungi, but reside in Kingdom Stramenopila with diatoms and brown algae. They are currently classified in the Peronosporomycetes within the Oomycota, although phylogenetic relationships within this group remain in question. *Phytophthora* has a wide host range and the damage caused by *Phytophthora* on crops like potato, black pepper, cardamom, coconut, arecanut, cocoa, citrus, vegetable crops like chillies, capsicum, tomato, fruit crops such as papaya, guava, passion fruit etc. result in economically significant yield losses. *Phytophthora* can infect all parts of a plant, including the roots, stems, leaves and fruit at any stage of growth, and can be seed borne, surviving in the soil and on host debris for months. But infection most commonly occurs during periods of heavy rainfall and high humidity in plantings that are over-crowded, over-fertilized with nitrogen or where poor drainage or excessive irrigation occurs. *Phytophthora* diseases are difficult to control 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.

The genomes of three *Phytophthora* were recently completely sequenced resulting in novel insights on the evolution and pathogenesis of Oomycetes. Whole-genome sequencing of oomycete species began with *Phytophthora sojae* (soybean root rot pathogen; 95 Mb genome) and *Phytophthora ramorum* (sudden oak death pathogen; 65 Mb genome) because they have smaller and simpler genomes than the 240 Mb *P. infestans.* There remains a high degree of conserved synteny among all three genomes, and a core set of some 9,500 orthologous genes is present in each of the species. The total number of genes annotated for each genome is similar, ranging from 14,451 (*P. ramorum*) to 16,988 (*P. sojae*) and 17,797 (*P. infestans*). The application of molecular and DNA sequencing analyses has altered our view of oomycetes and provided new insight into the evolution of these organisms and how they have excelled as plant pathogens.

SIGNIFICANT ACHIEVEMENTS FOR THE YEAR 2009-10

1

INDIAN INSTITUTE OF SPICES RESEARCH, CALICUT

Principal Investigator : **Dr. M. Anandaraj**
Co-investigators : Dr. K. Nirmalbabu, Dr. Johnson K. George, Dr. R.S. Bhai and Dr. Santhosh J. Eapen

Activities and achievements

1. Collection, conservation and maintenance of *P. capsici* and nematodes

- *Phytophthora* isolates (241 isolates) maintained in the repository have been revived and was enriched with 43 new *Phytophthora* isolates collected from different parts of Kerala and Coorg district of Karnataka, bringing the total to 284 (Table 6).
- Twenty new *R. similis* isolates and 11 root knot nematodes were isolated from 82 samples collected from different parts of Kerala. *R. similis* isolates were maintained on carrot discs and also on black pepper rooted cuttings. *Meloidogyne* spp. were maintained on tomato.

Table 6. Details of *Phytophthora* isolates maintained in the repository

Sl.No.	Host plant	No. of isolates revived from the repository	No. of new isolates	Total
1	Betel vine	24	-	24
2	Capsicum	02	-	02
3	Cardamom	04	03	07
4	Citrus	10	02	12
5	Cocoa	07	01	08
6	Coconut	07	01	08
7	Colocasia	09	-	09
8	Crossandra	02	-	02
9	Gerbera	01	01	02
10	Nutmeg	03	-	03
11	Pepper	126	33	159
12	Periwinkle	03	-	03
13	Rubber	08	-	08
14	Strawberry	03	-	03
15	Tomato	11	-	11
16	Vanilla	03	-	03
17	Miscellaneous	18	02	20
	TOTAL	**241**	**43**	**284**

2. Studies on diversity of *P. capsici* isolates using biological markers

- Metalaxyl sensitivity of *Phytophthora* isolates was studied for 10 isolates and mating type determination was done for 87 isolates. Majority of the isolates (55 Nos.) belong to A1 mating type.
- DNA was isolated from 126 isolates and SSR profiling is in progress with 25 pairs of specially designed primers. ITS region of *R. similis* was amplified with universal primers.

3. Species specific/strain specific detection and quantification in host tissue and soil

- Species specific markers for detection and quantification of *P. capsici* and *R. similis* have been designed and employed (Fig.1).

4. Host-pathogen-environment interactions

- Rhizosphere fungi and bacteria were isolated black pepper from rhizosphere and their antagonism against *P. capsici* was tested. Out of the 74 fungal isolates, five isolates showed antagonism against *P. capsici* and out of 94 bacterial isolates, eight showed antagonism.

5. Identification and cloning of AVR genes, resistance genes and transcription factors

- Putative *Phytophthora* isolates containing *elicitin* genes were identified through custom designed primers which amplified a product of 220 bp (Fig. 2). Cloning and sequencing of the amplified product is in progress.
- A protocol for isolation of RNA was standardized and further refined using the acid–phenol guanidinium thiocyanate method. DDRT-PCR experiment is in progress.
- Eight pairs of degenerate primers were designed from conserved p-loop and GLPL motifs of NBS- LRR regions of six

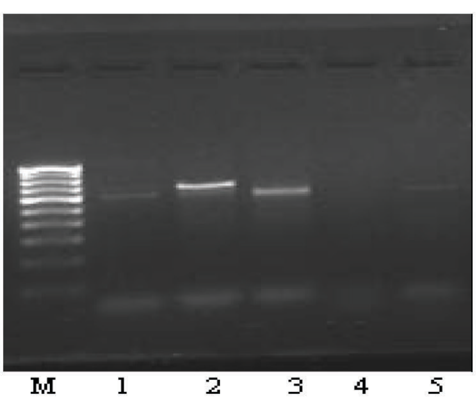

Fig. 1a. Amplification of ITS region of *Radopholus similis* using universal primers. M-100 base pair ladder, 1-5 *R. similis* and 6-negative control

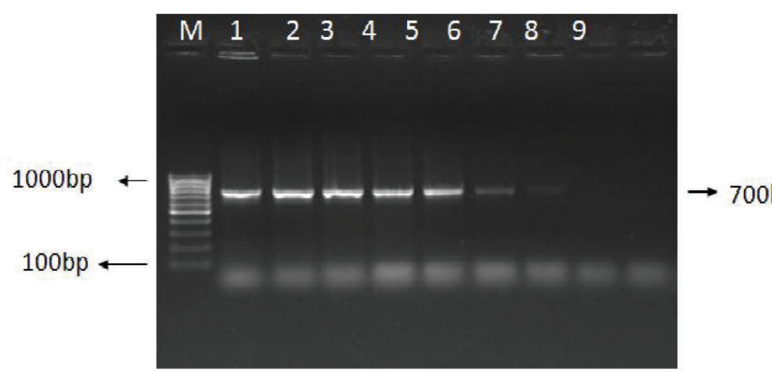

Fig. 1b. Amplification of ITS region of *Phytophthora capsici* using species specific primers. M-100bp ladder, Lane 1-7 *P. capsici* isolates, Lane 8 - *P. palmivora*, Lane 9 - *P. myriotylum*

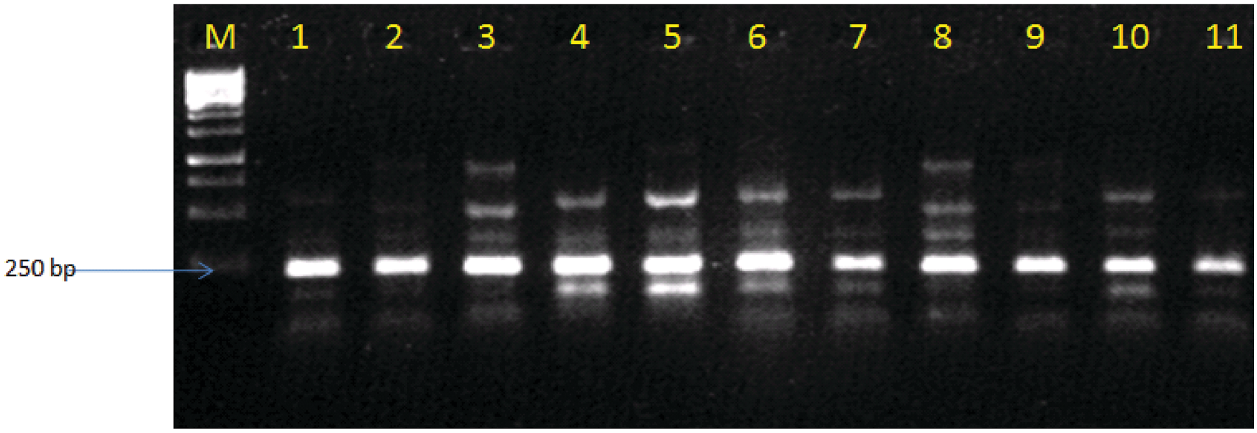

Fig. 2. Amplification of elicitin gene in *Phytophthora capsici* infecting black pepper M) Marker-1kb ladder; Highly virulent strains-Lane 1) 98-177, 2) 03-10, 3) 05-14, 4) 06-17; Moderately virulent strains-Lane 5) 96-03, 6) 96-01, 7) 96-10, 8) 98-185, 9) 05-03; Less virulent strains -Lane 10) 97-11, 11) 99-144.

Phytophthora resistance genes and R gene candidates was successfully amplified in black pepper (Fig. 3).

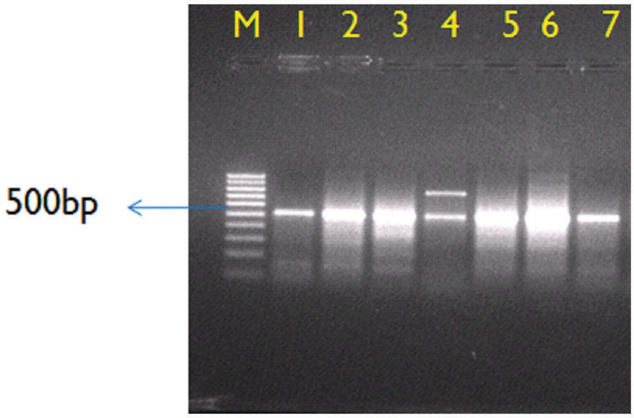

Fig. 3. Amplification of the R gene sequence in black pepper. M – Marker; 1 – P24; 2 – P24-o4-0; 3 – *P. colubrinum*, 4 – *P. ornatum*, 5 – IISR Sreekara, 6 - IISR Subhakara, 7 - Karimunda (bulk) 8 – Negative control

6. Development of molecular markers for screening and screening of parental lines

- Fifty parental lines for association mapping were screened for their reaction to *P. capsici* using leaf, stem and root inoculation techniques.
- Two scar markers developed for *Phytophthora* resistance were tested and validated in black pepper (Fig. 4).

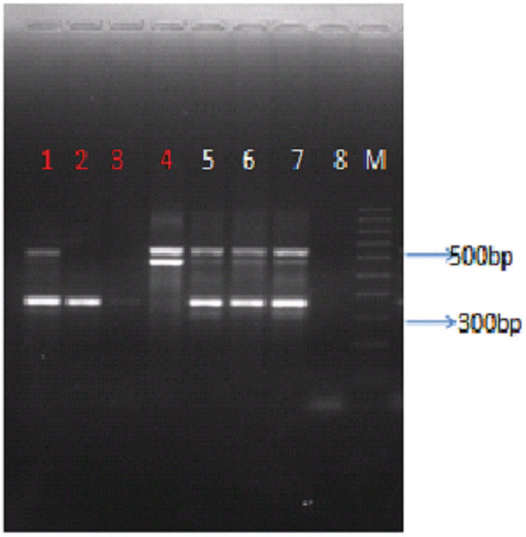

Fig. 4. Screening of SCAR markers for *Phytophthora* resistance in black pepper lines. 1) IISR Sakthi, 2)P24-O-4, 3)P. colubrinum, 4) P. ornatum, 5) IISR Subhakara, 6) IISR Sreekara, 7) Karimunda (bulk), 8) Negative control and M) Marker – 100 bp ladder.

7. Selection of parents for gene pyramiding and raising of segregating mapping populations

- Data on morphological characters were collected and DNA isolation was made from 57 lines of a mapping population (Panniyur 1 x Subhakara). Crosses were made between P24 X Subhakara for developing another mapping population. A selfing (OP) programme was done to develop Perambramundi -P24-P24 O4-progenies. Progenies of *P. colubrinum* segregating for *Phytophthora* resistance were developed (Fig. 5).

Fig. 5. Progenies of *Piper colubrinum*. (*Top row*) Seedling stage; (*bottom left*) Seedlings used for screening; (bottom right) leaf infection by *P. capsici*

- Two structured mapping populations were developed involving parental combinations of Panniyur 1 X Subhakara and P24 X Subhakara. Another population of about 50 lines segregating for *Phytophthora* resistance was also selected to characterize black pepper lines segregating for *Phytophthora* resistance and to tag the resistance with DNA markers. Multiplication of selected populations has been done. Genomic DNA isolation of 75 P1 X K1 progenies is completed and ISSR profiling is in progress.

8. Isolation and screening of endophytic fungi for biological control

- A total of 80 endophytic fungal isolates were recovered from different parts of black pepper. They were grown in malt extract agar for four weeks and characters such as colony diameter, shape, colour, elevation and effect on medium were recorded and tested for their antagonistic properties. Only 15 isolates were found to have antagonistic effect on the test pathogen *P. capsici* (Fig. 6).

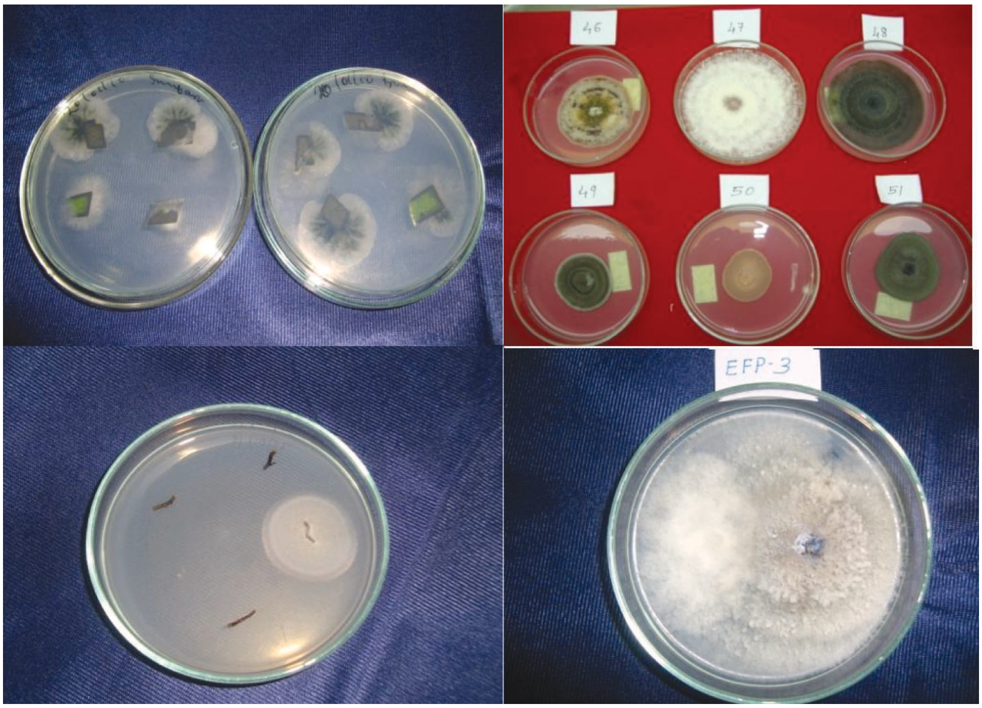

Fig. 6. Endophytic fungi isolated from black pepper. *Top left & bottom left* - Fungi isolated from root and leaf tissues; *Top right* – Characterization; *Bottom right* – Dual plate assay

9. Studies on planting material/soil health management

- Nursery experiments to study the effect of different soil disinfestations methods and soil health management using inorganic amendments have been initiated.
- Field trials on promising endophytic bacteria and resistant lines for the management of *P. capsici* and nematodes were continued.

10. Assembly and annotation of *P. capsici* ESTs

- Assembly and annotation of 56,457 expressed sequence tags (ESTs) of *P. capsici* was carried out. The cleaned sequences were assembled into 5966 contigs and functional annotation of contigs was performed using BLASTx from NCBI. Of this, 4885 contigs have homology to proteins with significant functions, around 17.8% ESTs were assigned to hypothetical proteins of unknown function, and 0.31% were designed as "no hit." The analysis of ESTs enabled to identify a range of genes likely to be involved in pathogenesis, drug resistance, stress, host degradation and genetic marker related protein for *Phytophthora* identification.

11. *In silico* identification of SSRs in *P. capsici*

- About 223 microsatellites were detected from 9831 UniGene sequences of

P.capsici and primers were designed for them. Majority of them (71.6%) were tri repeats.

12. Developing PHYTOWEB portal for database creation and monitoring

- PhytoWeb, a comprehensive portal on *Phytophthora* diseases of horticultural crops in India was developed by modifying the existing PhyDisH (Fig. 11). Phytolib, an electronic database of research publications on *Phytophthora* has also been developed and launched through this portal.

A web interface for monitoring the PhytoFuRa project on a real time basis was also developed (Fig. 12). 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 also view the compiled periodic progress reports, financial statements etc. It also has the facility to send the published papers online.

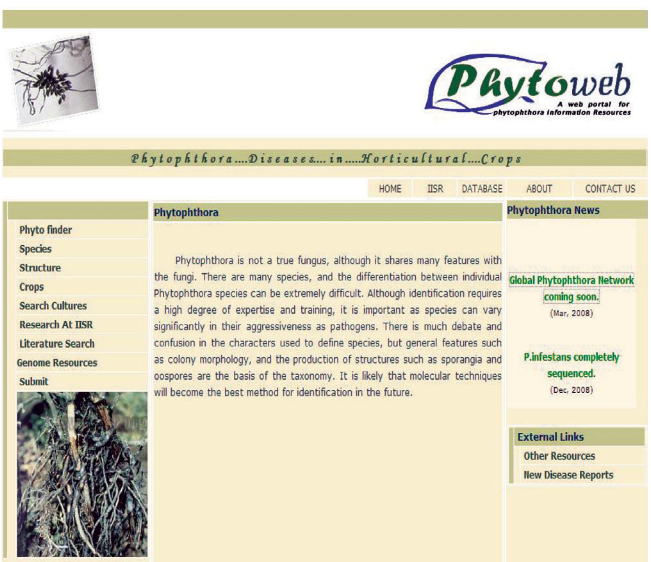

Fig. 7. Home page of Phytoweb, a portal on *Phytophthora* diseases of horticultural crops in India

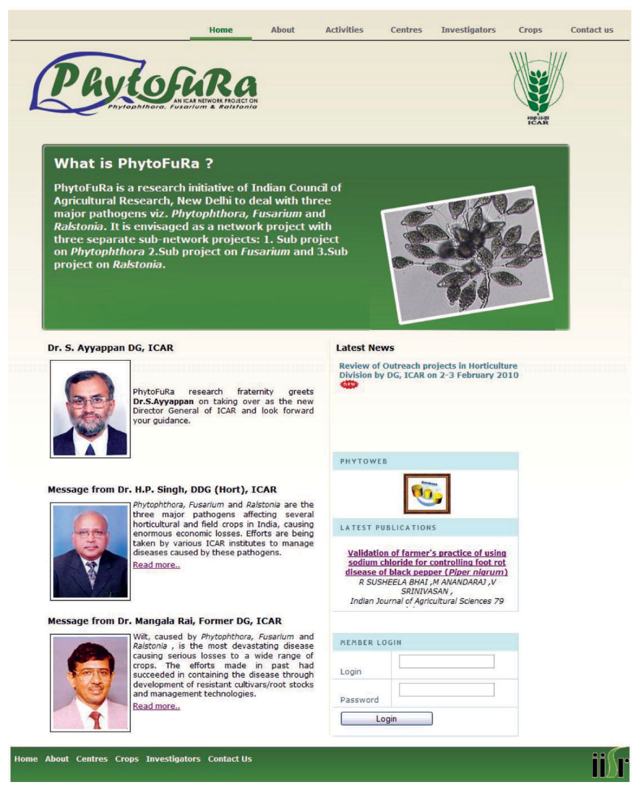

Fig. 8. The homepage of PhytoFuRa project

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CENTRAL PLANTATION CROPS RESEARCH INSTITUTE, KASARAGOD

Principal Investigator : **Dr. R. ChandraMohanan**
Co-investigators : Dr. K. Devakumar, Dr. N. Ravikumar

Activities and achievements

1. Survey and collection of *Phytophthora* isolates from different states of India

- Surveys were conducted in Kerala, Karnataka, Tamil Nadu and Andhra Pradesh for the incidence of bud rot of coconut. Among the disease endemic areas, the highest incidence of bud rot was observed in Kozhikode District of Kerala closely followed by Udupi District of Karnataka (Table 7).

Table 7. Occurrence of coconut bud rot disease in southern states

Sl. No.	State/District	No. of gardens surveyed	No. of gardens with bud rot/fruit rot	Intensity
KERALA				
1	Kasaragod	25	20	Severe
2	Kannur	38	25	Severe
3	Kozhikode	57	50	Severe
4	Wayanad	30	24	Severe
5	Thrissur	18	-	-
6	Malappuram	20	2	Sporadic
7	Palakkad	15	3	Sporadic
8	Ernakulam	25	8	Moderate
9	Idukki	25	8	Moderate
10	Kottayam	21	-	-
11	Pathanamthitta	23	-	-
12	Alappuzha	28	-	-
13	Kollam	22	-	-
14	Thiruvananthapuram	28	-	-

KARNATAKA				
1	Dakshina Kannada	17	6	Moderate
2	Udupi	19	16	Severe
TAMIL NADU				
1	Coimbatore	23	3	Sporadic
2	Kanyakumari	8	-	-
ANDHRA PRADESH				
1	West Godavari	32	-	-
2	East Godavari	30	13	Moderate

- bud rot not observed

- Among the *Phytophthora* diseases of cocoa, black pod and canker were observed as the major diseases. In nurseries, seedling dieback caused by *P. palmivora* was noticed as a serious problem. A total of 490 cocoa gardens were surveyed in Kerala, Karnataka, Tamil Nadu and Andhra Pradesh. Black pod disease was found occurring in 84.3% of the gardens surveyed. The highest incidence of stem canker was observed in Andhra Pradesh followed by Tamil Nadu (Table 8).

Table 8. Occurrence and distribution of *Phytophthora* diseases of cocoa in India

State / District	Total no. of gardens surveyed	No. & % of the gardens with disease incidence					
		Black pod		Stem canker		Chupon blight/ twig dieback	
		No. of gardens	% of gardens	No. of gardens	% of gardens	No. of gardens	% of gardens
KERALA							
Thiruvananthapuram	10	4	40	-	-	-	-
Kollam	5	-	-	-	-	-	-
Pathanamthitta	21	21	100	9	42.5	16	76.2
Alappuzha	16	14	87.5	7	43.7	7	43.7
Kottayam	47	46	97.5	38	97.9	31	65.9
Idukki	44	44	100	37	100	30	68.2
Ernakulam	49	47	95.9	33	95.9	9	18.4
Thrisur	12	12	100	9	100	-	-
Palaghat	10	8	80	3	80	3	30.0
Malappuram	14	10	71.4	5	71.4	2	14.3
Wayanad	5	5	100	3	100	1	20.0
Kozhikode	34	34	100	21	100	23	67.6
Kannur	14	12	85.7	1	85.7	-	-
Kasaragod	11	8	72.7	3	72.7	1	9.0
Total	**292**	**265**	**90.7**	**169**	**57.9**	**123**	**42.1**

KARANATAKA							
Dakshina Kannada	30	30	100	23	76.6	30	100
Coorg	11	8	72.7	1	9.0	5	45.4
Uduppi	11	11	100	4	36.4	8	72.7
Uttara Kannada	17	16	94.1	1	14.2	14	82.3
Chikkamagalooru	17	15	88.2	2	11.7	13	76.5
Shimoga	9	9	100	3	33.3	5	55.5
Mysore	10	10	100	-	-	3	30.0
Total	**105**	**99**	**94.2**	**34**	**32.3**	**78**	**74.3**
TAMIL NADU							
Coimbatore	23	16	69.5	21	91.3	4	17.3
Kanyakumari	8	8	100	2	25.0	2	25.0
Total	**31**	**24**	**77.4**	**23**	**74.2**	**6**	**19.3**
ANDHRA PRADESH							
East Godavari	32	17	53.1	32	100	6	18.7
West Godavari	30	11	36.6	30	100	3	10.0
Total	**62**	**28**	**45.1**	**62**	**100**	**9**	**14.5**
Total of four states	**490**	**416**	**84.9**	**288**	**58.8**	**216**	**44.1**

- A total of 136 isolates were collected from coconut bud rot and fruit rot disease endemic areas of southern states of India. Of these, 99 *Phytophthora* isolates were from bud rot samples and 37 isolates were from fruit rot affected samples. Among the *Phytophthora* isolates collected, 103 isolates were identified as *P. palmivora*.

- A total of 369 *Phytophthora* isolates causing diseases of cocoa were collected from different locations of South India. *Phytophthora* isolates were mainly collected from cocoa grown as mixed crop in existing coconut and arecanut gardens as well as from multispecies cropping systems involving spices, banana, rubber, coffee etc.

2. Cultural and morphological characterization of *Phytophthora* isolates

- Variations in cultural and morphological characters were observed among *P. palmivora* isolates from coconut. The rate of growth of the 56 isolates on carrot agar medium varied from 7.2 to 17.8 mm/day. Based on the rate of growth, the 56 isolates were grouped into three culture types. Variations were also observed in shape and pedicel length of sporangia. Pedicel length varied from 2.15 to 5.00 µm and the L/B ratio varied from 1.32 to 1.99.

- Studies on sporangial morphology of 35 isolates from coconut showed that the sporangia, in general, were ovoid to ellipsoid in shape with round base

Sporangial stalk was short, broad and occluded. Pedicel length varied from 2.15 to 5.00 µm. Based on cultural and morphological characters, the 35 isolates from coconut were identified as *P. palmivora*.

- Studies on cultural characters of 283 isolates from cocoa on carrot agar medium revealed that majority of the isolates exhibited stellate/striate/combed pattern of growth. Rate of growth of the isolates (283) varied from 7.2 to 17.8 mm/ day. Based on the rate of growth the isolates were grouped into two culture types. Studies on the sporangial morphology of 103 isolates revealed that they were mainly ovoid to ellipsoidal in shape with a round base and conspicuous papilla. Sporangial stalk was broad, short and occluded in all the isolates (pedicel length: 2.20 to 3.89 µm). Based on cultural and morphological characters 103 isolates were identified as *P. palmivora.*

3. Studies on pathogenic variability

- Variability was also observed in the virulence of 35 isolates of *P. palmivora* of coconut. The isolate KL-CO/32 from Kasaragod District of Kerala was found to be the most virulent isolate. Studies on other *Phytophthora* isolates of coconut are in progress.
- Studies on pathogenic variability of 80 *P. palmivora* isolates from cocoa indicated that there is variation in the virulence of the isolates. Among the 80 isolates, the most virulent *P. palmivora* isolate (KL-CA/ 125) was isolated from Idukki District of Kerala State. Studies on other *Phytophthora* isolates are in progress.

3

CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA

Principal Investigator : **Dr. B. P. Singh**
Co-investigators : Dr. Mehi Lal, Dr. Surinder Kumar Kaushik, Dr. S. K. Chakrabarti, Dr. Debasis Patanayak, Mohammad Alimuddin Khan

Activities and achievements

1. Collection, maintenance and DNA isolation of *P. infestans*

- Sixty isolates of *Phytophthora infestans* were isolated from different geographic locations of India and maintained on Rye Agar Media. Among them 22 isolates were from HP hills, 8 from Karnataka, 3 from Pantnagar and 27 from Indo-gangetic plains. Isolation of DNA from 40 isolates has been completed by using Qiagen Kit.

2. Studies on diversity of new *P. infestans* isolates using biological markers

- Studies on mating type revealed that Hill isolates (H.P. hills) are of only A2 mating type whereas in the sub-tropical plains, the A2 mating type was either absent (Bihar, Punjab, West Bengal) or its frequency was low (up to 12%) as in U.P. and Karntataka. The decline in the effectiveness of metalaxyl-based compounds was observed in the current crop season also. Tolerance of *P.infestans* population varied in different states viz. U.P. and Karnataka - 54% at 200 ppm and 22 % at 400 ppm, H.P. Hills - 87 % at 200 ppm and 39% at 300 ppm, Punjab - 41% at 200 ppm, West Bengal - 10 % at 150 ppm and Bihar - 23.5 % at 150 ppm.

3. Development of Decision Support System (DSS) for late blight

- JHULCAST model has been validated during December 2009-Janauary 2010. According to JHULCAST model forecasting has been done for the critical appearance of late blight. The predicted probable date was 6-10 January and the actual appearance occured on 7 January 2010. Weather data have been collected for development of DSS.

4. RNAi-mediated silencing of *RXLR* and *CRN* effector genes of *P. infestans*

- One important *P. infestans* gene having *RXLR* motif, *Avr3a*, which is involved in pathogen virulence by suppression of host cell death, was targeted for development of RNAi gene construct by putting the gene sequence in inverted repeat orientation intervened by an intron sequence.

5. Artifical microRNA-mediated silencing of *Phytophthora infestans* avirulence gene for resistance against the pathogen

- Five amiRNAs having complimentarity against five different regions of *Avr3a* mRNA and without any off-targets were selected for amiRNA construct development with the help of Web MicroRNA Designer tool platform (http://wmd3.weigelworld.org) (Table 9).

Table 9. *Avr3a* amiRNA sequences and their target regions in the *Avr3a* mRNA

Sl. No.	*Avr3a* amiRNA (5’’!3’)	Target region on *Avr3a* mRNA	Target gene (5’’!3’)	amiRNA reverse complement/1-21	Remarks
1.	TTT ACC CAT AAG CTG TTT CGC	262-282	GCG AAA CAG CTT ATG GGT AAT	GCG AAA CAG CTT ATG GGT AAA	One mismatch at 52 -end position one
2.	TAT GTA GTG AGC TGG CGT CGC	91-111	GGG ACG CCA GCT CAC TAC ATA	GCG ACG CCA GCT CAC TAC ATA	One mismatch at 52 -end position 20
3.	TTG GTT TGG TCG ATT GCG CTG	56-76	CAG TGC AAT CGA CCA AAC CAA	CAG CGC AAT CGA CCA AAC CAA	One mismatch at 52 -end position 18
4.	TTC TGG TCT AGC GTA ACC CTA	324-344	CAG GGT TAC GCT AGA CCA GAT	TAG GGT TAC GCT AGA CCA GAA	Two mismatches at 52 -end position 1 and 21
5.	TTC TGA TTG TAC TTT GCG CGT	381-401	AGG CGC AAA GTA CAA TCA GAT	ACG CGC AAA GTA CAA TCA GAA	Two mismatches at 52 -end position 1 and 20

6. Acquisition of molecular markers tightly linked to R genes, synthesis and validation of PCR primers

- DNA was extracted from 21 late blight differentials with known R-gene background. In addition, DNA was also extracted from 44 Indian potato varieties and 43 advanced hybrids emanating from various breeding programmes of the institute. Primers were synthesized for markers SPUD 237 and R1AS (R1gene) and cLET5E4 and GP 185 (R3a gene) (Table 11) and the process for their validation in late blight differentials has been initiated.

Table 10. Specific primers designed for R genes

S. No.	Name	Primer sequence (5’ – 3’)
1	SPUD 237 F	TTC CTG CTG ATA CTG ACT AGA AAA CC
2	SPUD 237 R	AGC CAA GGA AAA GCT AGC ATC CAA G
5	R1AS F	CAC TCG TGA CAT ATC CTC ACT A
6	R1AS R	CAA CCC TGG CAT GCC ACG
7	cLET5E4 F	CCA GGC ATG CTC AAT TTG GAG T
8	cLET5E4 R	TTC CCT GTT TGG ACT ACT TGT GGA
9	GP 185 F	CTG GTA ATA GTA GTA ATG ATT CTT CGT C
10	GP 185 R	TTG TTC AAT GGA GCA CTT GC

7. Validation of available SSR markers, DNA isolation and genotyping using SSR markers

- All the 126 F1 offspring of the cross *S. spegazzinii x S. chacoense* were maintained *in vitro* as micro plants. The population was genotyped using 4 AFLP primer combinations and 1 SSR primer set and a molecular map of *S. chacoense* developed using 134 markers (Fig.9).

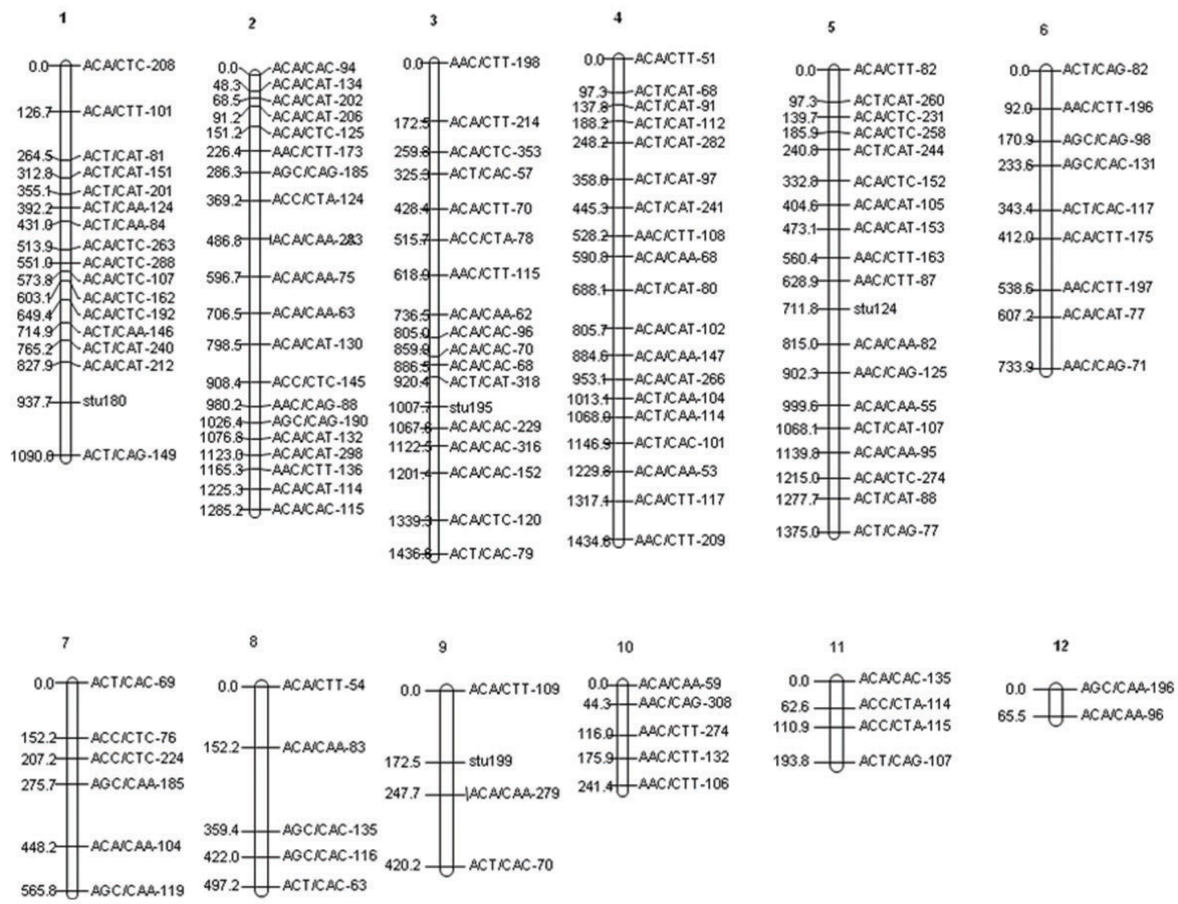


Fig. 9. A molecular map of *Solanum chacoense* generated using AFLP and SSR markers

8. Isolation of novel microorganism with activity against *P.infestans*

- Twenty five bacterial isolates have been isolated and some of them showed >33% emulsification activity and others showed <33% emulsification activity. In dual culture test one bacterial isolate showed positive effect on *P. infestans* and it was identified as *Pseudomonas* sp.

9. Effect of elevated temperature on host resistance, fungicide efficacy and aggression on *P. infestans*

- Effect of temperature on efficacy of fungicides viz. Acrobat, Curzate (Cymoxanil + Mancozeb) Mancozeb and Ridomyl (Metalaxyl + Mancozeb) were tested at 25 °C.

4

CENTRAL TUBER CROPS RESEARCH INSTITUTE, THIRUVANANTHAPURAM

Principal Investigator : **Dr. R.S. Misra**
Co-investigators : Dr. Vinayaka Hegde and Dr. M L Jeeva
Activities and achievements

1. Collection, conservation and characterization of *Phytophthora* isolates

- *P. colocasiae* was isolated from mature leaves of taro showing typical symptoms of taro blight from Kerala and North Eastern states of India. A total of 15 pure cultures were added to the culture repository along with the eight cultures obtained from IISR, Calicut. Morphological and cultural characterization of the isolates was completed.

2. Morphological and cultural characterization

- Three different media viz. Potato Dextrose Agar (PDA), Carrot Agar (CA) and V8 Juice Agar were tested to find out the best medium suited for the optimum growth of *P. colocasiae.* Among the solid media tested, CA supported the maximum mycelial growth followed by PDA for all the *P.colocasiae* isolates tested**.** The isolates differed in their colony characteristics on culture media.
- Effect of various temperatures viz. 26˚C, 28˚C, 30˚C, 32˚C and 34˚C on mycelial growth of *P. colocasiae* was monitored on two well supported growth media viz. PDA and CA. Among the various temperatures teste, 28˚C favoured the maximum growth rate (2.8 cm) of *P. colocasiae* except for the isolate P11.

3. Isolation of elicitor from *Phytophthora colocasiae*

- Cell wall glucan elicitor of *P. colocasiae* was isolated and its elicitation activity was studied by assaying the â -1, 3- glucanase activity in two months old taro plants.
- Cell wall lipid of *P. colocasiae* was isolated and elicitation activity was studied by injecting the crude lipid concentrate in to the main vein of third leaf of two months old taro plants. Higher percentage of necrosis was observed in the leaf injected with crude lipid after 72hrs when compared to control. Purification and further identification of the lipid elicitor fraction is in progress.

4. Identification of suitable promoter for elicitor gene isolated from *Phytophthora colocasiae*

- Genomic DNA was isolated from young leaf tissues of taro (*Colocasia esculenta*, var. Sree Resmi and Sree Kiran). Attempts to amplify the wound inducible promoter PVS3 using specific primers yielded an amplified product of about 750 bp.

5. Preparation of bioformulation against tuber rot and leaf blight

- The biocontrol agent *Trichoderma harizanum* having antagonistic activity against *P. colocasiae* was mass multiplied on organic materials like neem cake and wheat bran in the ratio of 1: 2.
- Spores of *Trichoderma* were mixed with talc, vermiculite, talc + wheat bran (5:1) and vermiculite + wheat bran (5:1) and their shelf life was studied. It was found that the presence of wheat bran has increased the spore count in both talc and vermiculite mixes by 2 and 1 fold when compared to their individual mixes.

5

DR. Y.S. PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY, KULLU

Principal Investigator : **Dr. I.M. Sharma**
Co-investigators : Dr. D. Gupta, Dr. Kishore Khosla,
Dr. Bupesh Gupta, Dr. P. Gupta and Dr. Rajinder Kaur

Activities and achievements

1. Survey and collection of Phytophthora isolates

- Survey was conducted in different apple orchards located in districts of Kullu, Mandi, Shimla and Kinnaur for the incidence of collar rot disease (*Phytophthora cactorum*) of apple. The incidence of the disease varied between 2.1-77.5, 0.8-20.6, 0.6-9.2 and 0.2- 5.8 per cent, respectively. Maximum incidence of the disease was observed in apple orchard at Johal in Anni block and Karjan in Naggar block of Kullu District, Ruhmini in Mandi, Tikkar - Pujarli in Shimla and Lippa in Kinnaur districts. In total 310 soil samples were collected from different apple orchards.
- Different methods such as baiting (apple fruit, guava fruit, tomato leaf etc.) and standard procedures were tried to isolate *Phytophthora* from soil samples. Finally, a method using deodar needles was developed to isolate the target pathogen.
- A total of 72 different isolates of *Phytophthora* have been isolated from the soil samples collected from Kullu and Mandi orchard soil. Ten isolates have been isolated from apple soil of Shimla District.

2. Effect of temperature on *Phytophthora*

- An experiment on the effect of temperature on incubation and lesion size using excised twig method indicated that a temperature of 25°C was conducive for the disease development and produced the symptoms within three days of incubation.

3. Screening of rootstocks of apple against *Phytophthora cactorum*

- Screening of 11 apple rootstocks against *P. cactorum* by exicised twig method indicated that rootstock M9, M26, *Malus prunifolia* and *M. floribunda* were highly resistant whereas MM106 and MM104 were highly susceptible. Similarly among different apple cultivars cv. Vance Delicious was highly resistant, Royal Delicious moderately resistant and Wellspur, Starking Delicious, Granny Smith were highly susceptible.

4. Isolation and evaluation of biocontrol agents

- Thirty two fungal and 23 bacterial antagonists have been isolated from the soil collected from different locations in four major apple growing districts of the state by using standard procedures.

- *In vitro* evaluation of these antagonists indicated that *Trichoderma harzianum* Is. 6 and Is. 15, *T. viride* Is. 5 and Is. 23 and *T. virens* Is. 2 were highly effective. Similarly, *Bacillus* Is. 3, Is. 11 and Is. 21 and *Pseudomonas* Is. 6 and Is. 14 were effective against the target pathogen.

5. *In vitro* evaluation of plant water extracts against *P. cactorum*

- *In vitro* screening of twenty water extracts of leaf/seed of different plants indicated that 20% leaf water extracts of *Vitex negundo*, *Melia azadirachta*, *Xanthoxylum esculantum*, *Brassica oleracea*, *Murraya koningii* and castor showed significant growth inhibition after eight days of incubation at 20.5±2 °C.

6. Effect of soil solarization on disease control

- Soil solarisation using 25, 50 and 75 µm thick, transparent polyethylene sheets during June - August 2009 for 45, 60 and 75 days increased the soil temperature by 9.2–13.7°C and prevented the soil moisture loss up to an extent of 52.6-64.6 per cent.
- Bits of actively growing culture of target pathogen kept at different soil depths (5–30cm) were killed up to 25 cm soil depths below the sheet on solarization.
- Stratified seeds of apple sown in pots having solarized soil had 89.6 per cent less disease incidence.

6

ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

Principal Investigator	:	**Dr. Ram Dutta**
Co-investigators	:	Dr. T.K. Bag

Activities and achievements

1. Survey, isolation and characterization of *Phytophthora* spp. from different agro-climatic zones

- Twenty two isolates of *Phytophthora* were collected from citrus growing belts of Meghalaya, Tinsukia District of Assam and Lohit District of Arunachal Pradesh and preserved in the laboratory for further studies.

2. Isolation and characterization of antagonistic microorganisms

- Isolates of *Pseudomonas fluorescens* were collected from rhizosphere soil samples of healthy tomato, maize, soybean, groundnut and rice plants from fields subjected to various cultural practices.
- Isolation of *Trichoderma* and *Gliocladium* from healthy citrus soil is going on using PDA media modified with antibiotics.

7

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT INSECTS, BANGALORE

Principal Investigator : **Dr. S. Sriram**
Co-investigators : Dr. R. Rangeshwaran and Dr. B. Ramanujam

Activities and achievements

1. Identification of *Trichoderma* isolates that elicit ISR

- To identify the *Trichoderma* isolates that elicit Induced Systemic Resistance (ISR) in chilli plants, the colorimeteric assays for induction of peroxidase, phenylalanine ammonia lyase and glucanase were carried out. Thirteen *T. harzianum*, 25 *T. viride* and 10 *T. virens* isolates that are maintained in the institute were screened for their ability to induce the peroxidase and phenol content in chilli plants by root dip treatment with their talc formulation. Further the biochemical changes in the phenol and protein contents were also assayed. Specifically, *T. harzianum i*solates Th 7, Th 19, Th 16, Th 10, *T.virens* isolates TVS 7, TVS 8, TVS 1, and *T.viride*isolates TV 5, TV 12, TV 22, TV 30, TV 97, TV 10 have been selected based on their ability to induce phenol and peroxidase in chilli plants on treating with talc formulation of these isolates.

2. Isolation, purification and characterization of elicitors from *Trichoderma* spp.

- *T. harzianum* isolates have been screened for the glucanase induction in chilli plants. Isolates Th-8, Th-1, Th-9 and Th 10 were found to induce more glucanase activity in plants.
- Glucan elicitors were extracted from *T. harzianum* isolate PDBC Th-10 that has good biocontrol potential and tested for their capacity to induce systemic resistance in red pepper plants.
- Elicitor preparations obtained from cell walls of *T. harzianum* significantly induced the glucanase activity in the leaves of red pepper plants when plants were treated with elicitor. The phenol content also increased in plants treated with glucan cell wall elicitors (Fig. 13 a & b).
- Treatment with elicitor (filtrate) preparation significantly reduced the infection by *P. capsici* in red pepper when treatment was given as seedling dip and plants were maintained in the sick soil infected by the pathogen.

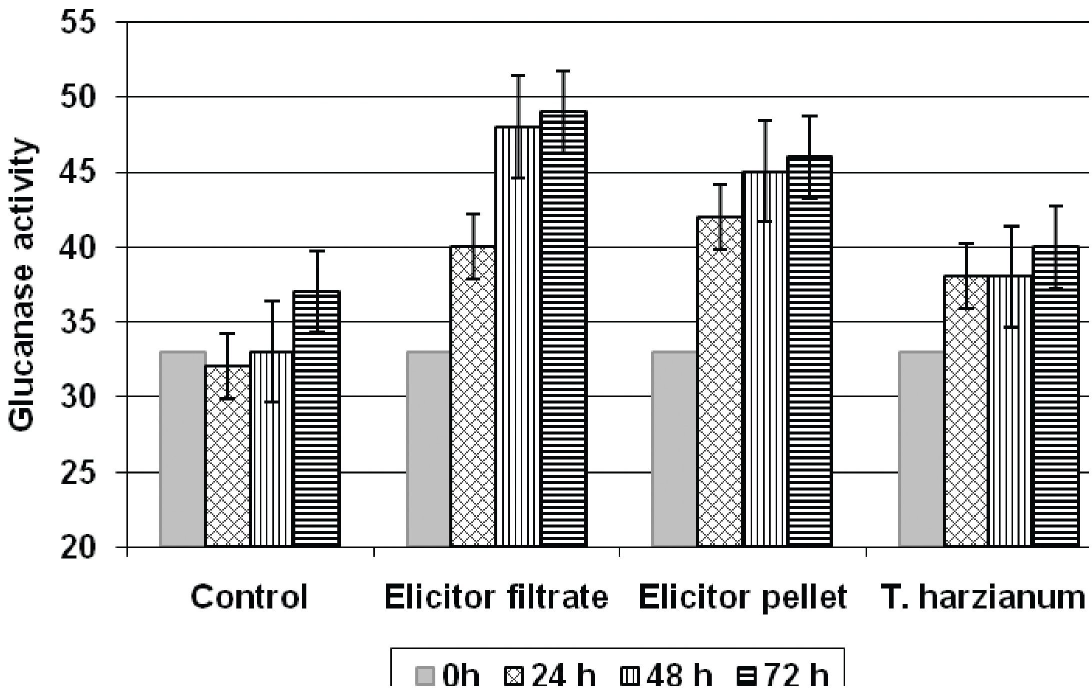


Fig.10a. Glucanase activity (μg of glucose released per min per g of leaf tissue) in red pepper plants treated with *T. harzianum* (talc formulation) and elicitor preparations (cell wall glucans) from *T. harzianum* (PDBCTH10)

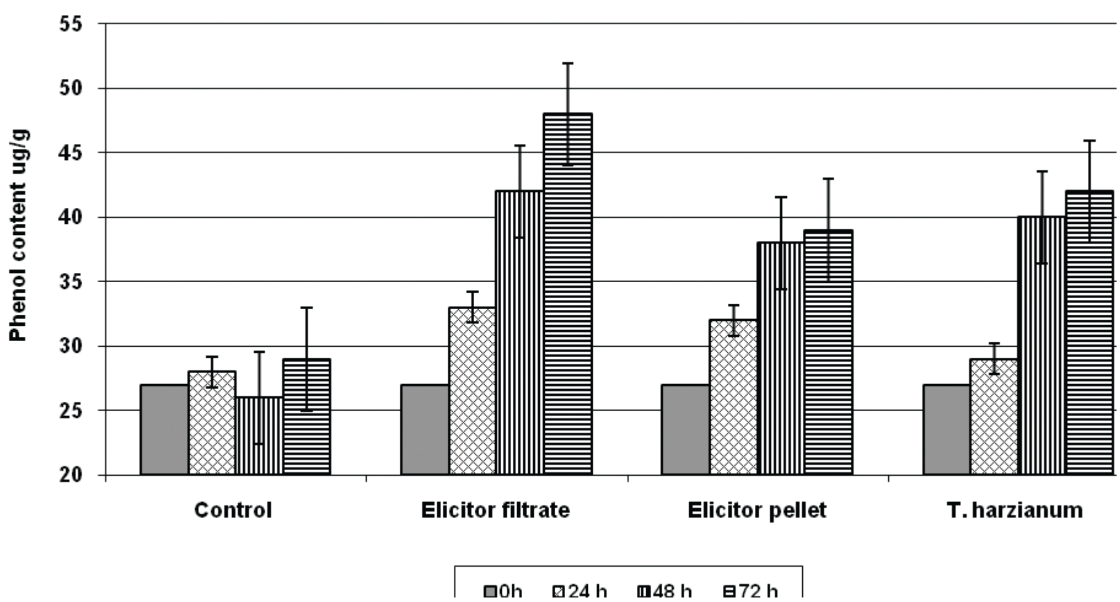


Fig.10b. Phenol content in red pepper plants treated with *T. harzianum* (talc formulation) and elicitor preparations (cell wall glucans) from *T. harzianum* (PDBCTH10)

8

NATIONAL RESEARCH CENTRE FOR CITRUS, NAGPUR

Principal Investigator	:	**Dr. A. K. Das**
Co-investigators	:	Dr. I. P. Singh and Sh. Vikas Bamel

Activities and achievements

1. Collection, conservation and diversity analysis of *Phytophthora* spp. isolates

- Surveys conducted in Vidarbha region of Maharashtra and Chhindwara District of Madhyapradesh revealed that *Phytophthora nicotianae* and *P. palmivora* are widespread causing gummosis, foot rot and root rot diseases leading to severe decline and death of Nagpur mandarin plants (Fig. 11a-d).

Fig. 11a: Gummosis symptoms on a branch of Nagpur mandarin tree

Fig. 11b: Extensive *Phytophthora* foot rot lesion on a Nagpur mandarin tree showing bark death

Fig. 11c: Root rot of Nagpur mandarin tree grafted on rough lemon root stock

Fig. 11d: Decline of Nagpur mandarin tree due to Phytophthora Foot rot and gummosis

- Seventy nine isolates of *Phytophthora* spp. (64 isolates of *P. nicotianae* and 15 isolates of *P. palmivora*) causing diseases in citrus were collected and maintained in sterile distilled water. Another set of cultures is maintained in Corn Meal Agar (CMA) plates also.
- Five different media viz. carrot agar (CA), V8 juice agar (V8), corn meal agar (CMA), lima bean agar (LBA) and potato dextrose agar (PDA) were evaluated for the growth of *Phytophthora nicotianae* and *P. palmivora* isolates. The isolates of both the species grew faster in CA and CMA than in V8, LBA and PDA. The colony of *P. nicotianae* isolates showed dense cottony mycelium to cottony aerial mycelium with no specific pattern of growth whereas *P. palmivora* isolates produced a stellate striated pattern colony on V8 agar media.
- Studies on sporangium morphology have revealed that *P. nicotianae* isolates produced more rounded pear shaped sporangia that were noncaducous, whereas *P. palmivora* isolates produced ovoid sporangia (mostly on sympodial sporangi-ophores) that were caducous but with short pedicel.
- Mating studies of 20 isolates with the known A1 mating type of *Phytophthora nicotianae* (ATCC-MYA-4036) revealed that only one isolate (NRCPh 18) belongs to A2 mating type while the rest are A1 type.

2. Development of molecular diagnostic methodology

- DNA extraction protocol from the mycelium of *Phytophthora nicotianae* and *P. palmivora* was standardized and the ITS region was amplified with the primer pair ITS 4 and 6. The ITS sequences had ~99% identity with *P. nicotianae* and *P. palmivora* (Fig. 12).

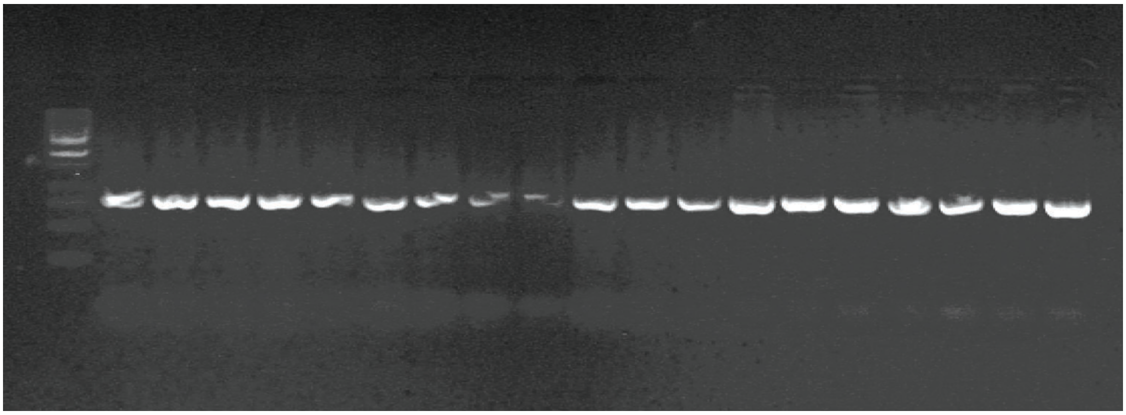


Fig. 12. PCR products amplified with primer pair ITS 4 and 6. Lane 1-14 : Isolate nos. NRCPh 1-14. M, 1 kb marker

- PCR-RFLP analysis of the extracted DNA (with the restriction enzymes *Msp*I and *Alu*I) of *Phytophthora* spp isolates using ITS primers confirmed the morphological assessments of the collected isolates as *Phytophthora nicotianae* and *P. palmivora*.
- Primer pair ITS3 – PNIC1 was designed for specific detection of *P. nicotianae* isolates. PCR amplification of genomic DNAs using this primer pair generated a 455 bp sequence for isolate no. 1-8 confirming that the pathogens were *P. nicotianae* (Fig. 13).

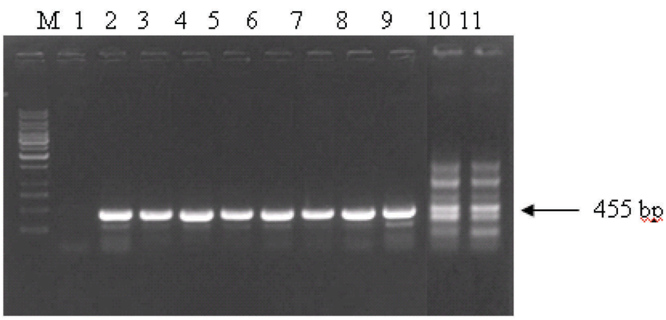


Fig 13. Agarose gel electrophoresis of PCR products obtained with *P. nicotianae*-specific primers (ITS3 – PNIC1). Lane 1, blank, lane 2-9, Isolate nos. NRCPh1-8, lane 10-11, Isolate nos. NRCPh13-14 M, 1 kb marker.

- Genetic diversity studies through RAPD analysis of nine isolates of *P. nicotianae* (NRCPh 1-9) showed that isolates NRCPh 7, 8 and 9 formed a different cluster from the rest of the isolates.
- *Phytophthora nicotianae* and *P. palmivora* were detected in citrus roots using nested PCR and PCR-RFLP technique (Fig. 14).

3. Screening of citrus rootstocks to evaluate resistance against *Phytophthora* spp.

- Out of the six rootstocks screened for root rot resistance, Rough lemon x Trifoliate hybrid was found moderately susceptible whereas rest all were found highly susceptible.

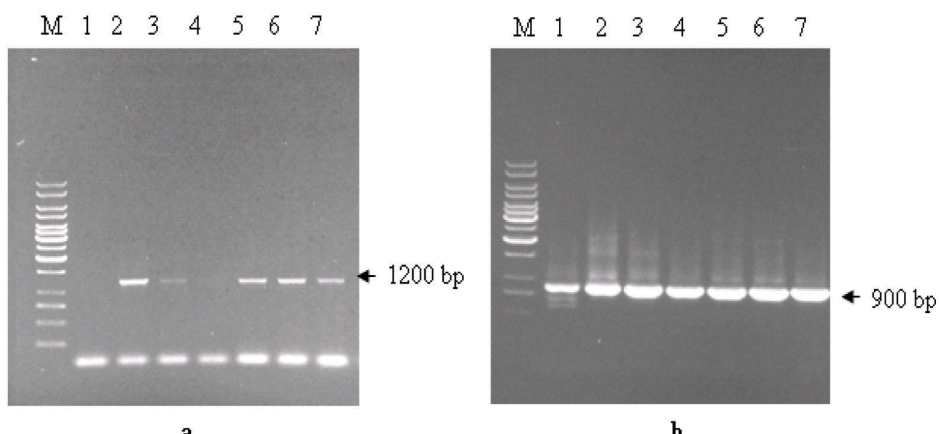


Fig 14. Amplification of DNA from infected citrus roots. (a) Simple PCR with primers DC6 and ITS4 (b) Nested PCR with primers ITS4 and ITS6. Lane 1-7: Root samples collected from infected Nagpur mandarin trees. M, 1 kb marker.

4. Search for novel bioagents and testing promising bioagents against *Phytophthora* spp.

- Fifty one nos. of fungal bioagents (mostly *Trichoderma* spp.) and 19 nos. of bacterial bioagents have been isolated and purified from citrus rhizosphere for their antagonism against *Phytophthora nicotianae*.
- *In vitro* efficacy studies of fungal bioagents revealed that none of the *Trichoderma* spp. isolates could develop any inhibition zone against the growth of *P. nicotianae* after 15 days but *Trichoderma* spp. overgrew on *P. nicotianae* growth on CMA. However, lysis of *P. nicotianae* mycelium was noticed caused by 17 isolates of *Trichoderma* spp.
- Promising 17 isolates of *Trichoderma* spp. were identified using PCR based molecular tools.
- The isolate NRCfBA -43 and –44 (*T. harzianum*) showed maximum root rot reduction against *P. nicotianae* in *in vivo* tests as compared to control.

SUBPROJECT - 2
FUSARIUM

The genus *Fusarium* collectively represents the most important group of fungal plant pathogens, causing various diseases on nearly every economically important plant species. Wilt diseases by *Fusarium* spp. is one of the most serious disease problems of several agricultural, vegetable and fruit crops. *Fusarium* is a large genus of filamentous fungus widely distributed in soil, plants, animals, arthropods and humans. Though most species are harmless saprobes and are relatively abundant members of the soil microbial community, a few of them, particularly the members of *F. oxysporum* Schlecht. Emend. Snyder and Hansen are responsible for devastating vascular wilt disease in plants of over 100 cultivated plant species, including important crops such as tomato, potato, sugarcane, bean, cowpea, date and oil palm, as well as cooking and dessert bananas. The host range of these fungi is extremely broad, including a range of both gymnosperms and angiosperms. While collectively, plant pathogenic *F. oxysporum* strains have a broad host range; individual isolates usually cause disease only on a narrow range of plant species which has led to the idea of "special form" or forma speciales in *F. oxysporum*. New races of the pathogen continue to evolve, overcoming deployed resistance and thwarting plant breeding efforts. Of equal concern is the health hazard posed to humans and livestock by the plethora of *Fusarium* mycotoxins. The genus is known to secrete fumonisin mycotoxins in maize and trichothecene & estrogenic mycotoxins in cereals (*F. verticilloides* and *F.graminearum*). Besides their economic importance, species of *Fusarium* also serve as key model organisms for biological and evolutionary research. So far genome sequences (both chromosomal and mitochondrial) of *F. graminearum*, *F. verticilloides* and *F. oxysporum* have been published. Comparative genome analysis revealed that the number of genes is more in *F. oxysporum* than other two species besides its larger genome size.

SIGNIFICANT ACHIEVEMENTS FOR THE YEAR 2009-10

1

CENTRAL INSTITUTE OF SUB-TROPICAL HORTICULTURE, LUCKNOW

Principal Investigator	:	**Dr. B. K. Pandey**
Co-investigators	:	Dr. Muthu Kumar M.

Activities and achievements

1. Collection of pathogenic isolates, bioagents and associated nematode fauna from different guava growing areas

- A total of 145 isolates of *Fusarium* spp. comprising 84 *F. solani* isolates and 58 *F. oxysporum* isolates were collected from different guava growing areas of India which included 95 isolates from Uttar Pradesh, 16 from Madhya Pradesh, three from West Bengal, five from Chandigarh, eight from Punjab, 10 from Jharkhand, five from Rajasthan, two from Karnataka and two from Orissa.

2. Studies on cultural, morphological and pathogenic variability in different isolates

- All the 145 isolates were compared for cultural and morphological characteristics such as formation of micro & macro-conidia and pigment production.
- On the basis of colony colour and texture *F. solani* and *F. oxysporum,* isolates were grouped into six groups viz. pinkish white, white colony turning slightly purple, white cottony, yellowish brown, white pinkish and reddish colonies.

3. Molecular characterization of potential Fusarial isolates and identification of strain specific sequence in the genome

- PCR conditions were optimized using the basic protocol and the DNA was amplified using nine RAPD primers.
- Primers were designed for amplifying the ITS regions of *F. oxysporum* and *F. solani* using bioinformatics tools. All *Fusarium* isolates amplified a product of approximately 570 bp size. ITS-PCR-RFLP based strain characterization was standardized. Restriction digestion using Eco RI or Taq I indicated that the ITS regions of the *Fusarium* isolates contained recognition sites for these enzymes (Fig. 15).
- All *F. oxysporum* isolates amplified approximately 450 bp using translation elongation factor gene specific primer pair. This species specific primer pair amplified only *F. oxysporum* isolates but not amplified the *F. solani.*

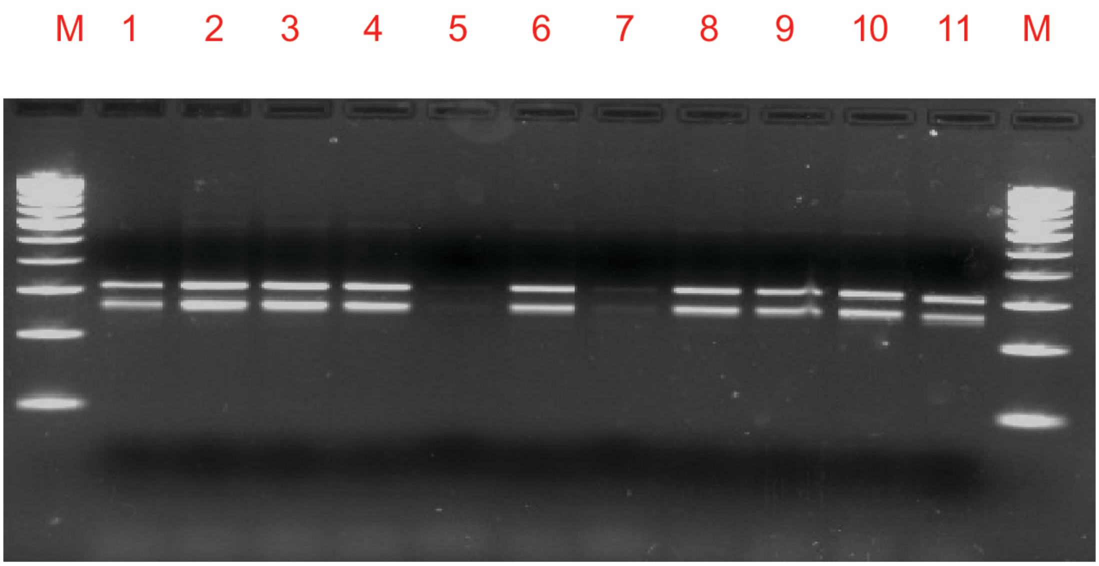


Fig 15: Restriction patterns of ITS regions of *Fusarium* isolates from different guava cultivars, digested with EcoRI.

4. Developing forewarning system for guava wilt

Weather data on temperature (maximum and minimum), relative humidity (maximum and minimum), rainfall and sun shine hours are being recorded periodically for developing forewarning system.

5. Enumeration of total microbial population from guava orchards from different location

Microbial analysis of soil samples collected from three different locations before and after the onset of rains indicated the abundance of *Bacillus, Pseudomonas* and *Fusarium solani*.

6. Studies on host resistance in guava

Different *Psidium* species, varieties and a hybrid (*Psidium molle x P. guajava*) were inoculated during the month of July - August with *F. oxysporum* using stem hole inoculation technique. Except hybrid *Psidium molle X P. guajava,* all other lines developed symptoms within 20 to 90 days.

7. Management of guava wilt

A field trail has been laid out for control of guava wilt by application of bioagents viz. *Aspergillus niger, Trichoderma harzianum, T. viride* and *Pseudomonas fluorescence*. Except two plants died in control, all the other plants are remaining healthy.

2

DIRECTORATE OF OILSEEDS RESEARCH, HYDERABAD

Principal Investigator : **Dr. R. D. Prasad**
Co-investigators : Dr. K. Anjani, Dr. S. Chander Rao and H. H. Kumara swamy

Activities and achievements

1. Diversity analysis of *Fusarium oxysporum* f. sp. *carthami using* molecular methods

- Based on the RAPD profile-based similarity coefficient driven cluster analysis, 51 geographical isolates of *Fusarium oxysporum* f. sp. *carthami* were grouped into five main groups. However, principal coordinate analysis based on similarity coefficient values identified only three major groups.
- Microsatellite analysis using ISSR primers grouped 51 isolates of *F. oxysporum carthami* from safflower into three genetic groups.
- Using the markers specific to the flanking region of the gene encoding the transcription elongation factor TEF-1-alpha, amplicons were obtained from 54 different geographical isolates of *F. oxysporum carthami* from safflower and these are under the process of cloning and sequencing.

2. Development of RT-PCR protocol for detection of *Fusarium* from plant sample

A CTAB method was optimized for the isolation of DNA from *Fusarium*. Real time PCR was developed for detection of *Fusarium* in wilted safflower. About 50 root samples of safflower were used to confirm the protocol.

3. Pathogenic variability in *F. oxysporum* f. sp. *carthami* isolates

Fifty-four isolates of *F. oxysporum* f. sp. *carthami* were tested for their pathogenic variability in five safflower lines *viz*. Nira, A1, DSF 4, DSF 5 and DSF-6. The reaction of 54 isolates to two differential hosts viz. DSF 4 and DSF 6 indicates existence of four races.

4. Marker assisted selection and molecular breeding in safflower

For marker assisted selection, five wilt resistant wild species *viz., C. creticus, C. oxyacantha, C. glaucus, C. lanatus* and *C. turkestanicus* were planted along with susceptible cultivated species *C. tinctorius* ('Nira') in a sick plot. All F_1s of the crosses between these and resistant parents were found resistant (0% wilt incidence).

A total of 777 RILs in F_6 generation of the cross between susceptible cultivated species (*C. tinctorius*) variety' Nira' and resistant line 96-508-2-90, planted in a sick plot, exhibited high resistance to wilt (% disease incidence). DNA samples from RILs were extracted to validate the already identified RAPDs linked to *Fusarium* wilt resistant gene in *C. tinctorius.*

3

INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Principal Investigator : **Dr. S. C. Dubey**
Co-investigators : Dr. Parimal Sinha

Activities and achievements

1. Collection, characterization documentation and conservation of *Fusarium*

- Seventy isolates of *F. oxysporum* f sp. *ciceris* causing chickpea wilt were collected from Delhi (2), Punjab (6), Haryana (3), Uttar Pradesh (9), Rajasthan (10), Jharkhand (6), Bihar (2), Madhya Pradesh (7), Chhatisgarh (3), Gujarat (4), Maharashtra (4), Andhra Pradesh (6) and Karnataka (8) and are being maintained for further studies.

2. Species /strain specific detection and quantification in host tissue and soil

- Genomic DNA was extracted from 20 isolates of *F. oxysporum* f sp. *ciceris*. One set of markers *Foc* F2 and *Foc* R2 (designed from the ITS sequences of *F. oxysporum* f sp. *ciceris*) was found suitable for detection of the population of *F. oxysporum* f sp. *ciceris*. The marker produced amplification of 292 bp specific to the *F. oxyspprum* f. sp. *ciceris* only (Fig. 16).

3. Evaluation of a new set of chickpea differentials against isolates of *F. oxysporum* f sp. *ciceris*

Virulence of 70 isolates of the pathogen was tested on a new set of 10 differential cultivars of chickpea, namely, C 104, JG 74, CPS 1, BG 212, WR 315, KWR 108, GPF 2, DCP 92-3, Chaffa and JG 62.

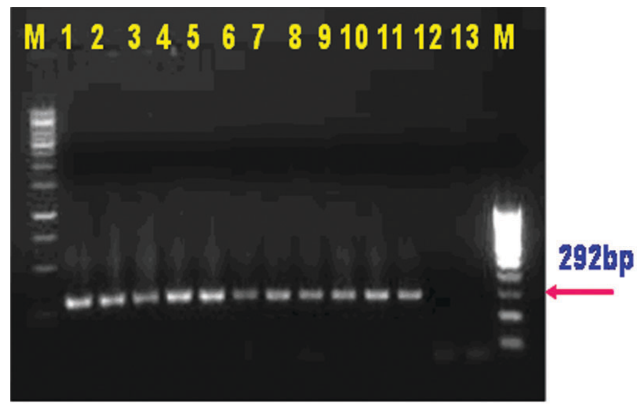


Fig.16. Amplified product of 11 different isolates with a set of markers FOC F2 and FOC R2 (Lane 1= (FOC 53) IARI, Delhi, 2= (FOC 97) Ranchi, Jharkhand, 3= (FOC 52) Gurdaspur, Punjab, 4= (FOC 62) Ropar, Punjab, 5= (FOC 20) Faridkot, Punjab, 6= (FOC 45) Ludhiana, Punjab, 7= (FOC 4) Jaipur, Rajasthan, 8= (FOC 42) Ganganagar, Punjab, 9= (FOC 41) Hisar, Haryana, 10=(FOC 50) Udaypur, Rajasthan, 11= (FOC 69) Sardargarh, Rajasthan, 12- *F. oxysporum* f. sp. *cucumerinum*, 13- *R. bataticola* and M - 1kb ladder at left and 100bp ladder at right sides)

4. Determination of tolerance in *T. harzianum* and *F. oxysporum* f. sp. *ciceris* to fungicides

Twelve fungicides at three concentrations were evaluated against *F. oxysporum* f sp. *ciceris* and *T. harzianum in vitro* using poisoned food technique. The results clearly indicated that amongst the fungicides, combined formulations of carboxin + TMTD, iprodion + carbendazim, and formulations of carbendazim, TMTD, metalaxyl, thiophanate methyl and propiconazole at all three concentrations tested inhibited cent percent growth of *F. oxysporum* f. sp. *ciceris* but were less inhibitory to *T. harzianum*. A combination of metalaxyl and mancozeb, captan and mancozeb alone caused variable inhibition to the pathogen but also less inhibitory to *T. harzianum*.

5. *In-vitro* efficacy of bacterial antagonists against *F. oxysporum* f. sp. *ciceris* in chick pea

Evaluation of three isolates of *Pseudomonas fluorescens*, namely Pf 59, Pf 62 and Pf 80 and two isolates of two different species of *Bacillus*, namely *B. lechniformis* (Bl) and *Bacillus* species (Bs-km5) against two isolates (Foc 53 and Foc 118) of *F. oxysporum* f. sp. *ciceris* by dual culture technique Among the isolates of *P. fluorescens* evaluated, Pf-80 caused significantly highest inhibition followed by Pf-62 and Pf-59.

6. Compatibility test among potential isolates of *T. harzianum,* PGPR and *Rhizobium ciceri*:

The compatibility of the most effective bacterial antagonists, namely, *P. fluorescens*-80, *Bacillus* species (Bs-Km-5), most effective isolate of *T. harzianum* and *Rhizobium ciceri* was tested amongst themselves. Amongst the two species of bacterial antagonists, only PGPR strain Pf-80 was found compatible with *T. harzianum* and *R. ciceri* with no inhibition zone.

4

INDIAN INSTITUTE PULSES RESEARCH, KANPUR

Principal Investigator : **Dr. Vishwa Dhar**
Co-investigators : Dr. R. G. Chaudhary and Dr. S. Datta

Activities and achievements

1. Collection, characterization documentation and conservation of *Fusarium*

- Ten isolates of *Fusarium udum* from Jalna, Akola and Ahmed Nagar districts of Maharashtra were collected and added to the repository of *F.udum* isolates. Similarly, *Foc* isolates from 27 morphological and pathogenic groups were isolated from different agroclimatic centres.

- The National Collection of 716 isolates of *F.udum* and 378 of *Foc* were characterized in to 91 and 58 cultural, morphological and pathogenic groups, respectively (Table 11). One isolate from each group representing different geographical areas was selected for studying pathogenic variability and for molecular characterization studies.

Table 11. Categorization of isolates of *Fusarium udum* and *F. oxysporum* f.sp. *ciceri* in to representative groups

No. of isolates	States	No. of districts	No. of representative groups
Fusarium udum **(Total No. of isolates = 716; No. of groups = 91)**			
229	UP and MP	48	31
91	Delhi, Haryana, Rajasthan and Punjab	25	18
138	Bihar, Jharkhand and West Bengal	25	17
254	Karnataka, Andhra Pradesh, Tamilnadu and Maharashtra	30	24
04	ICRISAT and Gujarat	02	01
F. oxysporum **f. sp.** ***ciceri*** **(Total No. of isolates = 378; No. of groups = 58)**			
103	UP, Karnataka and A.P.	45	19
126	MP, Chattisgarh, Gujarat and Maharashtra	62	25
149	Punjab, Haryana, Delhi, Jharkhand and Rajasthan	35	16

2. Diversity analysis *F.udum* and *F.oxysporum* f. sp. *ciceri* using SSRs

For molecular characterization and diversity analysis, DNA has been extracted from 24 isolates of *F.udum* and 36 isolates of *Foc* using CTAB method. Ten SSR markers were screened for standardizing the PCR conditions.

3. Identification and confirmation of variants of *F.udum* and Foc

Twenty four isolates of *F. udum* representing different states and 36 of *Foc* were inoculated on differential genotypes in pot experiments for identification of variants. Reactions of pigeon pea differential genotypes indicated prevalence of variant 1 of *F.udum* in Maharashtra, M.P. and Karnataka, variant 2 in Haryana, variants 1 & 3 in Rajasthan and variants 1 & 5 in A.P.

4. Identification and confirmation of variants of *F.udum* and Foc in pigeon pea and chick pea

Twenty nine sets of *Fusarium* specific primers (including primer pair ef 1 and ef 2 to amplify Translation Elongation Factor (TEF) gene region) have been synthesized for species and intra specific identification of *F.udum* and *F.oxysporum* f.sp. *ciceri* isolate.

5. Resistance screening of pigeon pea and chickpea genotypes

Different genotype of pigeon pea and chickpea comprising of wilt resistant donors, promising lines, national elite varieties, wilt differential genotypes, breeding lines and hybrid pigeon pea lines have been raised in a sick plot, predominantly having variant 2 of *F. udum* for evaluating their resistance to wilt.

6. PGPR and their role in disease management for pigeon pea /chick pea wilt

Seven promising antagonist strains identified through initial screening in lab by dual culture technique are being tested for their efficacy as seed treatment against *F.udum* and their growth promoting activities in three different doses (5, 10 and 15 /Kg seed) in a pot experiment. The data revealed that *T. viride* strain Dholi at 10 g/ kg seed followed by *T.harzianum* from Kanpur and *T.viride* from Bangalore were most efficient in enhancing the growth parameters and the number of *Rhizobium* nodules.

5

INDIAN INSTITUTE OF VEGETABLE RESEARCH, VARANASI

Principal Investigator	:	**Dr. M. Loganathan**
Co-investigators	:	Dr. S. Saha, Dr. Saritha R. K. and Dr. Venkattaravanappa

Activities and achievements

1. Survey, collection, documentation and maintenance of isolates of tomato wilt pathogen from different agro-climatic zones

- A survey was conducted in major tomato and chilli growing areas of Uttar Pradesh, Jammu & Kashmir, Haryana and Punjab, and collected the fusarial wilt infected plant samples. Nine *Fusarium* isolates from tomato and 34 isolates from chilli were isolated in pure form and are being maintained.

2. Characterization and diversity analysis of different isolates based on cultural, morphological and molecular characters

- The *Fusarium* isolates were characterized using cultural characteristics such as colony colour, pigmentation, mycelial growth pattern and morphological characters *viz*. hyphae, microconidia, macroconidia and chlamydospores were made for 62 fusarial wilt isolates. A clear cut difference could be noticed in macro conidia production among various isolates. Most the tomato isolates (*F. oxysporum* fsp. *lycopersici*) produced macro conidia which were absent in chilli isolates (*F. solani*/ *capsici*).

6

NATIONAL RESEARCH CENTRE FOR BANANA, TIRUCHIRAPALLY

Principal Investigator : **Dr. R. Thangavelu**
Co-investigators : Dr. S. Backiya Rani

Activities and achievements

1. Molecular characterization, Diversity analysis of *Fusarium oxysporum* f.sp. *cubense* using ISSR markers

- Molecular characterization was carried out for 180 isolates of *Foc* obtained from different parts of the banana growing regions of the country by ISSR analysis. Out of 18 primers screened against representative isolates of *Foc*, four primers viz., UBC 861, 825, 826 and 827 gave maximum polymorphic bands. Among these, the ISSR primer UBC 861 was used to characterize the 180 isolates of *Foc* and the result of the study based on phylogenetic analysis indicated that there are seven major groups present in *Foc* isolates of India.
- A study was conducted using ISSR markers to distinguish the *Foc* isolates based on the variety from which they were isolated. The virulent isolate of *Foc* from var. Robusta and Poovan was found to be the same through ISSR analysis. The *Foc* isolated from each variety also showed wide variation. This study clearly indicated that the effective management practices are also to be evolved depending on the strains present in each banana growing regions.

2. Designing degenerate primers and identification of suitable primers for banana

Resistant gene analogues (RGAs) of cultivar Rose were amplified using three primers namely S2/AS1, LRR F/ R and NBS/LRR primers and cloned into pGMT vector. The clones obtained from amplified product of S2/S1 primers have been randomly selected and is being sequenced.

The protocol for isolation of RNA from cv. Rose root samples was standardized.

3. Biological management of fusarial wilt of banana

Survey for isolation of bioagents: Twelve *Trichoderma* spp. isolates and eight isolates of rhizobacteria were isolated from 25 soil samples collected in a survey conducted in Tamil Nadu (Salem, Nammakal and Madurai - 20), Maharastra (5) and Tripura (10).

Non pathogenic Fusarium *isolates*: Thirty one isolates of non-pathogenic *Fusarium* were isolated from 22 different banana accessions resistant to *Foc* and these isolates were evaluated against *Foc* isolate 0124 for the inhibition of spore germination. Among these, three isolates recorded 100% inhibition of spore germination when compared to other isolates.

Endophytic bacteria: Eight endobacterial isolates having multiple actions were shortlisted from 185 isolates collected from 23 banana accessions resistant to *Fusarium* wilt. They were grouped in to nine different bacterial genera namely *Serratia, Staphylococcus, Klebsiella, Micrococcus, Bacillus, Citrobacter, Azotobacter* and

Pseudomonas based on morphological and biochemical tests. The DNA isolated from the effective endophytic bacteria were PCR amplified by using 16s rRNA universal primer, purified and being sequenced.

Endophytic Trichoderma *spp*.: Forty three isolates of endophytic *Trichoderma* spp. were isolated from 13 different *Foc* resistant banana germplasm. They belonged to *T. harzianum* (13 isolates), *T. viride* (29 isolates) and *T. pseudokoningii* (one isolate). On screening against *Foc* (VCG 0124) *in vitro* by eight different methods they were shortlisted into 12 isolates having multiple actions including phosphate solubilization.

Other *Trichoderma* spp.: Out of the 19 isolates screened, four isolates viz. *T. harzianum, T. pseudokoningii, T. koningii* and *T. viride* were found effective in either inhibiting the mycelial growth or spore germination of the pathogen.

Endophytic actinomycetes: Out of the 30 isolates of endophytic actinomycetes isolated from 20 different banana accessions resistant to Foc, seven strains of actinomycetes showed 100% inhibition of spore germination of *Foc* (Fig. 17).

In vivo *evaluation of bioagents against* Foc: Pot culture experiments conducted with six isolates of rhizospheric *Trichoderma* and one isolate of endophytic actinomycetes in different combinations against Foc 0124 indicated that application of Actinomycetes + *T. viride* K2T5 + *T. harzianum* recorded an internal score of 1.8 as against Foc score of 4.1 in the control (Foc alone inoculated plants) after four months of planting.

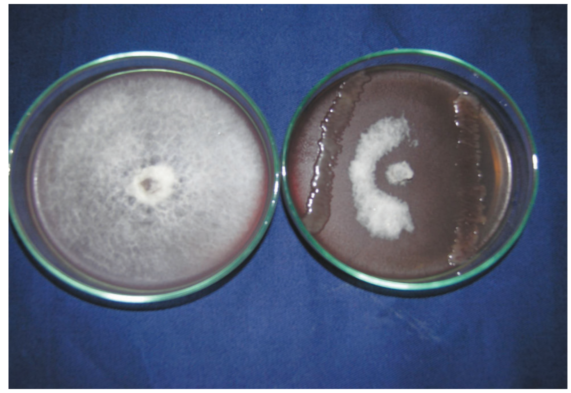


Fig.17. Inhibition of mycelium of *Fusarium oxysporum* f.sp. *cubense* (*Foc* 0124) by an effective actinomycetes strain under *in vitro* condition

4. Plant extracts against fusarial wilt of banana

Thirty three medicinal plant extracts were screened against *Foc* 0124 causing fusarial wilt in banana. Five plant extracts recorded 100% inhibition of spore germination and and among them only Zimmu leaf extract showed more than 1 cm zone of inhibition in agar well diffusion method.. The mycelial inhibition assay using Zimmu leaf extract through poison food technique also indicated that the zimmu leaf extract at 25 % conc. is effective in inhibiting (70%) mycelial growth of *Foc.*

7

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROBES, MAU

Principal Investigator : **Dr. Sudheer Kumar**
Co-investigators : Dr. D.K. Arora and Dr. Alok K. Srivastava

Activities and achievements

1. Collection and characterization of *Fusarium* cultures from different agro-climatic zones

- Nineteen isolates of *Fusarium oxysporum* f. sp. *lycopersici* received from Indian Institute of Vegetable Research, Varanasi (U.P.) have been preserved for short term (in mineral oil and in glycerol at -80^{0}C) as well as long term conservation.
- The *Fusarium* isolates 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. (Fig. 18). Between different isolates of *F. oxysporum* f. sp. *lycopersici* considerable variations were recorded in conidial size as well as growth, pigmentation and also formation of both intercalary and terminal chlamydospores.

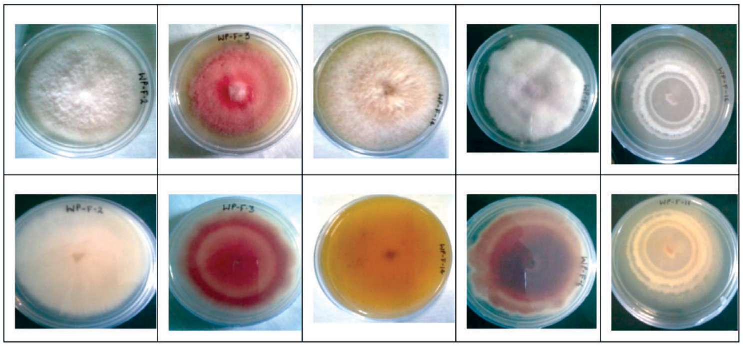


Fig.18. Variation in colony and pigmentation in different isolates of *F. oxysporum* f. sp. *lycopersici*

- Genomic DNA was isolated from different *Fusarium* isolates and the ITS1 and ITS4 region was amplified using ITS-PCR which yielded a fragment of 550 bp size (Fig. 19). No substantial polymorphic pattern was found among the isolates by using ITS with restriction enzyme Alu 1 on 2.5% agarose.

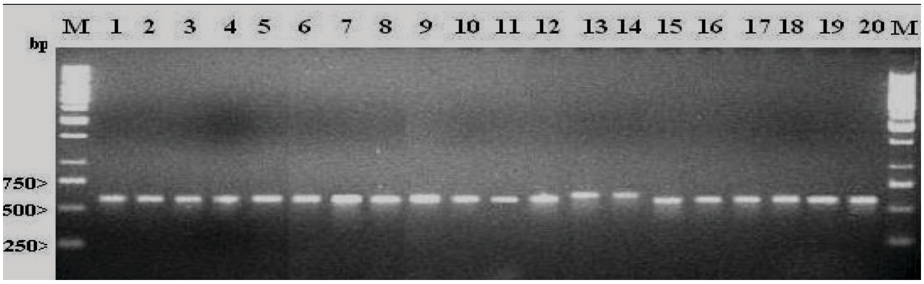


Fig. 19. Amplification of ITS region of *Fusarium oxysporum* f. sp. *lycopersici*

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT INSECTS, BANGALORE

Principal Investigator : **Dr. S. Sriram**
Co-investigators : Dr. R. Rangeshwaran and Dr. B. Ramanujam

1. Disease management using non pathogenic *Fusarium* isolates

Twenty-five non pathogenic *Fusarium* isolates were isolated and maintained from soil samples collected from rhizosphere regions of tomato plants grown in Karnataka, Tamil Nadu and Gujarat states of India. They were tested for pathogenicity using tomato (*Lycopersicon esculentum* Mill) cv. Selection 22 and host range using chick pea, pigeon pea, safflower, ground nut, castor, water melon and banana. The selected seven isolates of *Fusarium* spp viz. PDBC NPFu1, PDBC NPFu2, PDBC NPFu3, PDBC NPFu4, PDBC NPFu7, PDBC NPFu24 and PDBC NPFu25 were compared for variation in morphological and cultural characters and the identity of the isolates was confirmed at National Centre for Fungal Taxonomy, New Delhi. ITS-PCR of the selected isolates was carried out using the primers ITS1 (5' TCCGT TGGT GAAC CAG CGG 3') and ITS4 (5' TCCTCCGCTTATT GATA TGC 3') and the amplified products were sequenced.

The isolates PDBC NPFu4, PDBC NPFu3, PDBC NPFu24 and PDBC NPFu25 inhibited the growth of *F.oxysporum* f. sp. *lycopersici* by 32-40% and isolates PDBC NPFu1, PDBC NPFu2, PDBC NPFu3 and PDBC NPFu 7 showed 24- 27% inhibition in

Table 14. Growth of *Fusarium oxysporum* f. sp. l*ycopersici* and non-pathogenic isolates of *Fusarium* in dual plate culture

Isolate	Growth after 5 days (cm)			Growth after 7 days (cm)		
	NPF	FOL	% reduction in FOL over control	NPF	FOL	% reduction in FOL over control
Fu1	6.13	4.88	33.85	7.38	5.88	24.19
Fu2	3.63	4.63	37.25	4.25	5.63	27.42
Fu3	5.5	4.75	35.55	6.25	4.75	38.71
Fu4	5.00	4.25	42.33	6.25	5.25	32.26
Fu7	6.00	5.00	32.16	7.00	5.88	24.19
Fu24	5.00	5.5	32.16	5.65	4.88	37.10
Fu25	4.33	4.13	44.03	5.75	4.63	40.32
Pathogen	-	7.37	-	-	7.75	-

C.D @ 5% T- 5.00, F—9.3, T*F- 13.23.

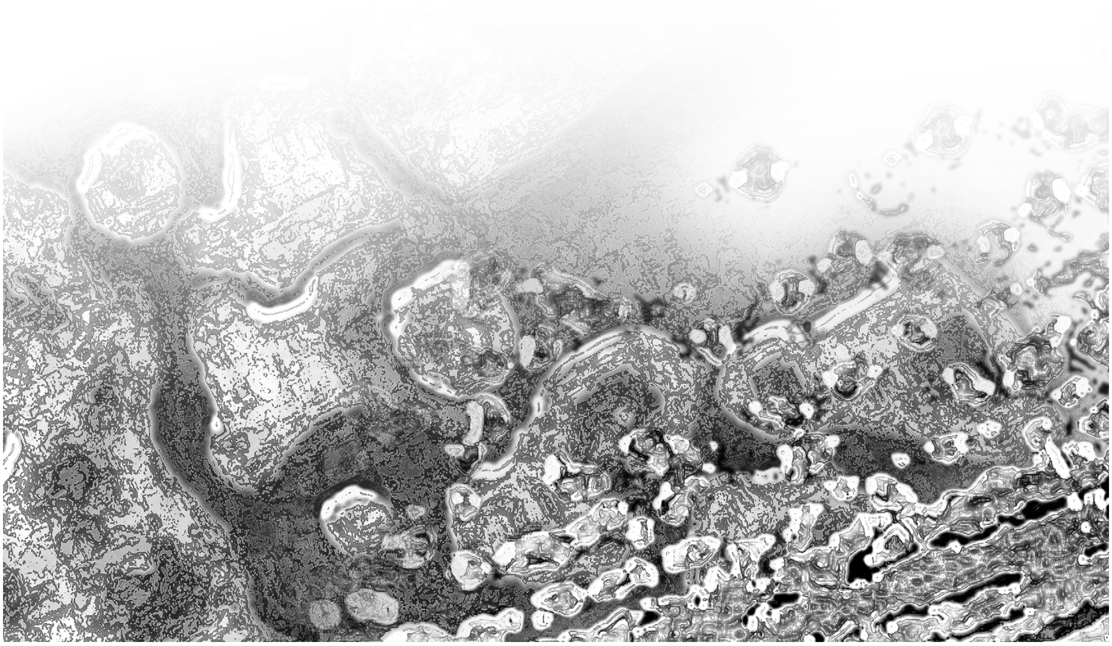
dual plate assays (Table 14). Over growth on pathogen by non-pathogen isolates was observed with the isolate PDBC NPFu 25. Incorporation of the selected isolates significantly increased the plant growth especially in shoot weight compared to control and no wilting symptoms were observed in any plants.

2. Induction of resistance by non pathogenic *Fusarium* isolates in tomato

Treatment with non-pathogenic *Fusarium* isolates on tomato induced peroxidase activity and contents of total phenol and total protein. There were no significant changes in poly phenol oxidase activity. The induction of biochemical defense mechanisms in tomato plants in response to treatment with non-pathogenic isolates of *Fusarium* spp. and respective elicitors was studied with different combinations of pathogen, non pathogen, elicitor, pathogen + non-pathogen, non-pathogen + pathogen, elicitor + pathogen, pathogen + elicitor and recorded for wilt incidence.

SUBPROJECT - 3

RALSTONIA



Ralstonia solanacearum Yabuuchi (Smith): a member of γ-proteobacteria is one of the most important and wide host range plant pathogens of crop plants reported to cause wilt disease in 200 plant species, representing over 50 botanical families and covering both monocots and dicots extending from annual plants to trees and shrubs. More recently, geographical distribution of the pathogen has been extended to more temperate countries from Europe and North America as the result of the dissemination of strains adapted to cooler climates. Worldwide, the most important crops affected are: potatoes, tomatoes, eggplant, chilli, ginger, tobacco, banana and Pelargonium. Knowledge gained over one hundred years of research on bacterial wilt reveals that the pathogen is extremely variable in nature with six biovars and five races has been reported. Recently phylotype-classification of *Ralstonia* confirmed existence of four distinct phylotypes with Asian, American, African and Indonesian origins. Disease spread is mainly by contaminated soil, surfaces, infected seed, irrigation and/or washes waters. *Ralstonia solanacearum* can survive in the soil and the infected land sometimes cannot be used again for susceptible crops for several years as experienced in ginger and potato. The diseases management options fails, owing to its multiple mode of survival in nature and to its high genomic diversity. Four full genome sequences have been published representing tomato and geranium hosts by various groups. Sequence data clearly suggest that the genome of the pathogen is bipartite with virulence genes are largely located in the megaplasmid, the second chromosome of the bacterium. The project under PhytoFuRa is aimed at deciphering the genetic diversity of *Ralstonia* collected from diverse geographical locations in Indian subcontinent and its management for increasing the crop productivity.

SIGNIFICANT ACHIEVEMENTS FOR THE YEAR 2009-10

1

INDIAN INSTITUTE OF SPICES RESEARCH, CALICUT

Principal Investigator : **Dr. A. Kumar**
Co-investigators : Dr. R. Suseela Bhai

Activities and achievements

1. Collection and maintenance of *Ralstonia* isolates

Isolates of *Ralstonia solanacearum* were isolated from bacterial wilt affected ginger plants collected from major ginger growing locations in India were preserved as glycerol stocks in -80°C & -20°C as well as in sterile water at 20°C. Present strength of the isolates is 86 representing hosts such as ginger, tomato, chilli, potato, and eupatorium.

2. Identification of race and biovar specific sequence in *Ralstonia solanacearum* using molecular characterization/ bioinformatics tools and development of diagnostic tools

Ralstonia solanacearum comprises of five biovars based on utilization/oxidation of carbon sources and five races based on the hosts they infect.

In a PCR-RFLP analysis of intergenic region between 16-23s rDNA of biovar 3 strains of diverse host origin *R. solanacearum* revealed number of biovar specific sequences (290bp) (Fig. 20). This sequence would be cloned and used as a probe.

3. Isolation of *R. solanacearum* from wilt affected ginger

Ginger plants expressing early symptoms of bacterial wilt were collected, subjected to ooze test and the bacterial ooze was streaked on to CPG agar for isolation of *R. solanacearum* (Fig 21). All isolates were found to be biovar-3 based on utilization/oxidation of carbon sources. The strains were found to possess poly beta hydroxybutric acid granules as probed by Nile blue staining (Fig. 22).

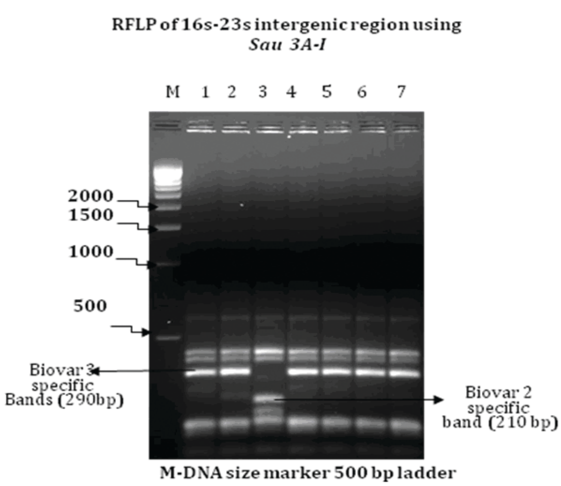
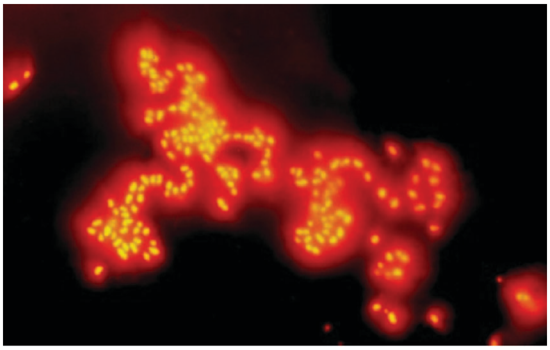


Fig. 20 RFLP analysis of 16S-23S intergenic region of *Ralstonia solanacearum* isolates using *Sau 3A-I*

Fig. 21. Typical colony of *R. solanacearum* (fluidal colony with spiral pink centre) CPG agar or Kelman's TZC agar



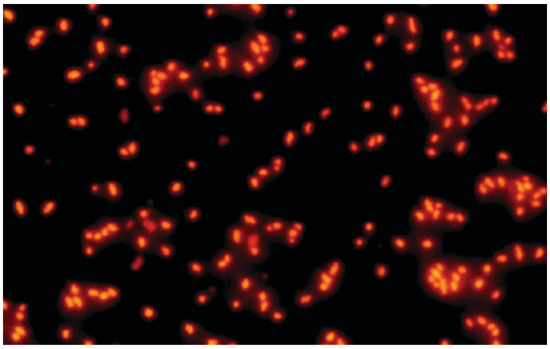


Fig. 22. Visualization of bacterial cells by staining of PBH granules using Nile

4. Assay for pathogenicity of *Ralstonia solanacearum* in ginger

For pathogenicity assay the bacterial colony picked from CPG medium was inoculated at the base of 30-45 days old ginger plants (3-4 leaf stage). The data on disease incidence and number of days to express wilt was recorded. The bacterium was reisolated from the wilted plants by adopting the protocol mentioned above.

5. Molecular characterization

PCR based confirmation of *Ralstonia solanacearum* was performed using universal primers (RsFP: 5'-GTC GCC GTC AAC TCA CTT TCC-3', RsRP: 5'- GTC GCC GTC AGC AAT GCG GAA TCG-3') suggested by Opina *et al,* (1997). Appearance of a single 281bp amplicon confirmed the identity of *Ralstonia solanacearum* (Fig. 23).

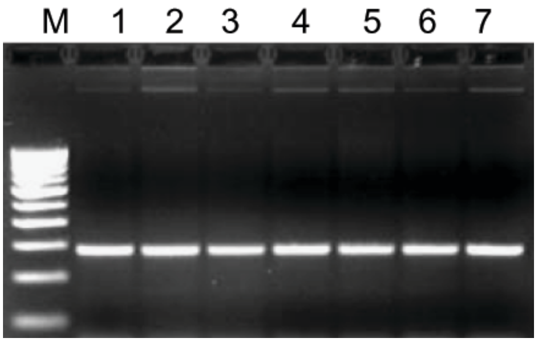


Fig. 23. PCR based identification of *Ralstonia solanacearum* Lane 1-7: Isolates of *Ralstonia solanacearum* from different host (281bp amplicon)

6. Gene coding for DNA repair protein

Sequences (1710bp) of gene coding for DNA repair protein, RecN sequences were extracted from fully sequenced strain GMI1000. The primers were validated for various parameters such as hairpin formation, self annealing and other physical & chemical qualities pertaining to the PCR .

7. Evaluation of rhizobacteria against *R. solanacearum*

Biological control strategies may either help development of alternative management measures or be integrated with other practices for effective bacterial wilt management at the field level. Altogether 51 bacterial isolates of rhizome bacteria were obtained from 28 samples collected from different ginger growing tracts and were maintained in nutrient agar slants. Out of 51 bacterial isolates checked, one bacterium from soil collected from Idukki showed signs of antagonism.

2

ICAR RESEARCH COMPLEX, GOA

Principal Investigator : **Dr. R. Ramesh**
Co-investigators : Dr. M. Thangam

Activities and achievements

1. Collection and characterization of *Ralstonia solanacearum* isolates

Ralstonia solanacearum isolates collected from brinjal, chilli and tomato from Goa (35 different places, 73 isolates); Karnataka (15 different places, 18 isolates), Kerala (13 different places, 27 isolates), Andaman Islands (25 places, 25 isolates) with the total of 181 *R. solanacearum* isolates are being maintained at the centre. Phenotypic characterization for biovar identification revealed the dominance of biovar 3 in India.

2. PCR based identification of *R. solanacearum* using specific primers

Universal *R. solanacearum* specific primer sets: OLI-1 & Y2 (288bp) (Seal *et al.*, 1993) and Rs759 & Rs760 (280 bp) (Opina *et al.*, 1997) were used for the confirmation of the identity of the isolates.

3. Analysis of genetic diversity of *R. solanacearum*

Phylotyping of the isolates was carried out using five primers (Fegan and Prior, 2005). One hundred and five isolates were tested from a total of 181 *R. solanacearum* isolates and the results indicated that all belong to phylotype 1.

4. Diagnostics

Detection threshold of *R. solanacearum* in the soil was standardized using PCR method. The minimum threshold limit ranged between 6.8X10 and 3.6X10^2 CFU/g of soil. Using this standardized protocol *R. solanacearum* was detected from the rhizosphere soil of eggplants showing varying degrees of wilt. Presence of *R. solanacearum* in the soil infested with capsicum wilt was also demonstrated successfully and the minimum detection limit was 4X10^2 CFU/g of soil. The bacterium was not detected from the eggplant seeds collected during 2006 and 2007 seasons. However, the bacterium was detected from the weeds grown in the eggplant field indicating the possible role of weeds serve as symptomless carrier.

5. Host resistance

Seeds (brinjal, tomato and chilli) have been obtained from GBPUAT, KAU, OUAT, NBPGR and IIHR. *Solanum torvum* seeds have been obtained from NBPGR. Forty four local accessions were obtained for screening for resistance. Screening for bacterial wilt resistance is in progress and three *R. solanacearum* isolates which differ in the virulence (highly, moderately and mildly virulent isolates) are used in the screening.

6. Isolation of microbial candidates for use against *R. solanacearum*

Study of xylem bacterial population: Endophytic bacteria including xylem residing bacteria were isolated and evaluated against bacterial wilt pathogen. Endophytic bacteria isolated from eggplant, cucumber and groundnut were evaluated against *R. solanacearum*. The plants treated with *Pseudomonas* isolates (EB9, EB67), *Enterobacter* isolates (EB44, EB89) and *Bacillus* isolates (EC4, EC13) reduced the wilt incidence by more than 70%. Most of the selected antagonists produced an antibiotic, DAPG, siderophore and IAA in the culture medium. Sixteen rhizobacterial isolates were selected based on their inhibition efficiency against *R. solanacearum*. Among the selected isolates, 75 per cent belonged to species of *Pseudomonas*, which was clustered into five phylogeny groups based on biochemical tests. The lowest wilt incidence (13.3%) was recorded in RC24 (*P. putida*) and in RBh1 (*B. subtilis*-20.0%).

7. Development of biological control methods and their validation

Talc formulation of two species of *Pseudomonas* (RBh41 and RBh42) completely suppressed the incidence of wilt up to 36 days of inoculation. Treatment with bacterial cells of *P. mallei* (RBG4, ET17) and one *Bacillus* spp. (RCh6) reduced wilt incidence of 83 per cent compared to control. *Pseudomonas* strains (RBh41, RBh42, RBG4, ET17) improved shoot and root length when eggplant seeds were treated.

3

ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

Principal Investigator	:	**Dr. Ram Dutta**
Co-investigators	:	Dr. T.K. Bag

Activities and achievements

1. Collection of *Ralstonia solanacearum* from ginger, tomato, chilli and brinjal

- A total of 66 *Ralstonia solanacearum* isolates were obtained from several location were phenotypically characterized, pathogenicity confirmed and preserved.

2. Isolation of microbial candidates for use against wilt pathogens

- Several rhizospheric and soil bacteria were isolated from diverse crop plants for use as a biocontrol agent.

4

INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Principal Investigator : **Dr. Dinesh Singh**
Co-investigators : Dr. K. K. Mondal

Activities and achievements

1. Diversity of *R. solanacearum* collected from different agro climatic zone

Ralstonia solanacearum causing bacterial wilt of tomato, brinjal, chilli, capsicum and potato was isolated from Himachal Pradesh, Jammu & Kashmir, Uttarakhand, Jharkhand and West Bengal states of India. A total 84 isolates of *R. solanacearum* were obtained and characterized by biological, cultural, biochemical and molecular methods. 61 isolates of *R. solanacearum* representing the states Jharkhand (15 isolates), Himachal Pradesh (13 isolates), Uttarakhand (29 isolates) West Bengal (3 isolates and Jammu & Kashmir (1 isolate) were further characterized using ERIC-PCR. Amplification products (15 fragments) ranging in size from 300 to 6000bp yielded fingerprint patterns. Among the 61 isolates 4 distinct genomic fingerprint genotypic clusters were identified. A 14 isolates were kept in cluster A, 34 isolates in cluster B, 13 isolates in cluster C and only one isolate in cluster D. Maximum population of *R. solanacearum* was found in cluster B which were isolated from different climatic conditions. In cluster D one isolate collected from potato which showed genomically entirely different from other groups.

2. PCR based identification of *R. solanacearum*

A primer pair of specific to *R. solanacearum* Y2 (5- CCCACTGCTGCCTCCCGTAGGAGT -3) and OLI1 5- GGGGGTAGCTTGCTACCTGCC-3) were used to identify *R. solanacearum*. All 27 isolates of *R. solanacearum* collected from states as J&K (1), Himachal Pradesh (8), Uttarakhand (10), Jharkhand (5) and West Bengal (3) could yield amplicon of 288bp from genomic DNA confirming their identity as *R. solanacearum*

3. Disease management

Species of *Pseudomonas* (8) and *Bacillus* (6) isolated from tomato and capsicum plants were found antagonistic against *R. solanacearum in vitro*. The most effective bacteria was identified as *Bacillus subtilis.*

5

INDIAN INSTITUTE OF HORTICULTURAL RESEARCH, BANGALORE

Principal Investigator : **Dr. C. Gopalakrishnan**
Co-investigators : Dr. M. Krishna Reddy

Activities and achievements

1. Collection and characterization of *Ralstonia solanacearum*

The major tomato, brinjal and chilli growing areas in Karnataka were surveyed for the collection of different isolates of *Ralstonia solanacearum.* About eighty *Ralstonia solanacearum* isolates were collected which include 23 isolates from tomato, five brinjal and seven chilli isolates. Pathogenicity of isolates was tested on tomato cultivar Arka Vikas. Eighteen days old seedlings were inoculated with RS isolates at concentration 1.0 x 10^8 cfu/ ml by leaf clipping method. All the isolates were found to be biovar 3 based on phenotypic characterization for biovar. The cultures are being maintained as glycerol stocks in -80C.

6

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT INSECTS, BANGALORE

Principal Investigator : **Dr. S. Sriram**
Co-investigators : Dr. R. Rangeshwaran and Dr. B .Ramanujam

Activities and achievements

1. Isolation, identification and evaluation of lytic phages against *Ralstonia solanacearum*

Phages specific for *Ralstonia solanacearum* was isolated from soil collected from various locations (Fig. 24). Well isolated plaques with different morphology (based on plaque size) were picked using sterile needles and purified by repeating the double agar layer method twice. To analyze the sensitivity of isolated phages to various pH, CPG broth with different pH ranging from 3-12 were prepared using 1N HCl or 1N NaOH. 100µl of diluted phage suspension was added to 0.9ml of broth and incubated for 1 h. After Incubation, the suspension was plated to check their stability at various pH. One step growth experiment to check the burst size of phage strains is also in progress. Although generally *R. solanacearum* do not produce pigments in CPG medium, a change in color of the medium to brown was observed in host culture infected with S6P2 phage isolate after 24hrs. Presence of pigments and loss of turbidity indicates that there may be possibility of pseudolysogeny (causing phenotypic conversion of host cell to produce pigments) followed by lytic life cycle.

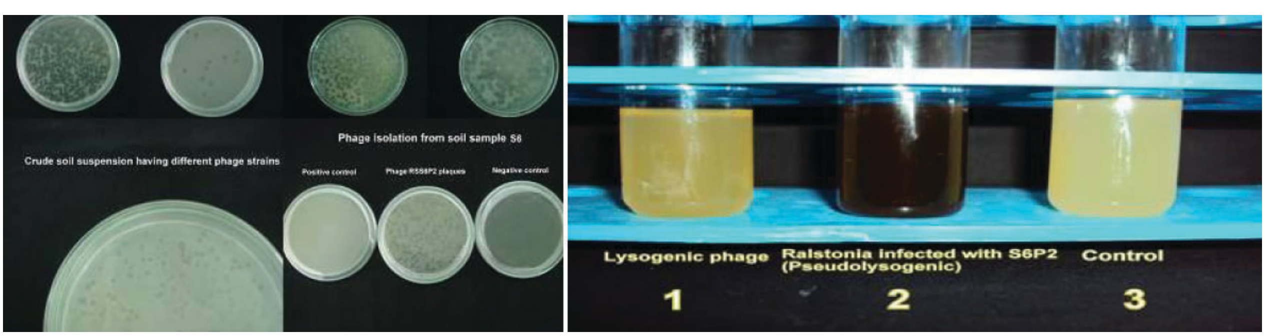


Fig. 24. Phages isolated from different soil samples

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1. Sharadraj, K.M. and ChandraMohanan, R. 2010. Status of bud rot disease of coconut in Kerala State. *Proceedings of 22nd Kerala Science Congress, 28-31 January, 2010*, KFRI, Peechi, pp. 63-64.

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BUDGET

Item	YEAR				Total
	2008-09 R E	2009-10 RE	2010-11 BE	2011-12 BE	
A. Recurring					
Contingencies	24.0	142.192	279.82	279.82	**725.832**
T A	1.0	0.0	63.0	60.0	**124.0**
H R D	0.0	0.0	100.0	59.0	**159.0**
B. Non Recurring					
Equipments	0.0	33.808	923.192	0.0	**957.0**
Total	**25.0**	**176.0**	**1366.012**	**398.82**	**1965.832**

BUDGET UTILIZATION

Head	RE sanctioned 2009-10	Funds released by ICAR so far	Prog. expd. Upto 31st jan 2010	% age expenditure against allocation/provision	% age expenditure against receipt
Outreach programme	176.00	75	135.00	76.70	180%