PhytoFuRa
AN ICAR OUTREACH PROJECT ON
Phytophthora, Fusarium & Ralstonia

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

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Annual Report 2012-13

भारतीय मसाला फसल अनुसंधान संस्थान
Indian Institute of Spices Research

Annual Report 2012-13

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

PhytofuRa
AN ICAR NETWORK PROJECT ON
Phytophthora, Fusarium & Ralstonia

ANNUAL REPORT 2012-13

INDIAN INSTITUTE OF SPICES RESEARCH
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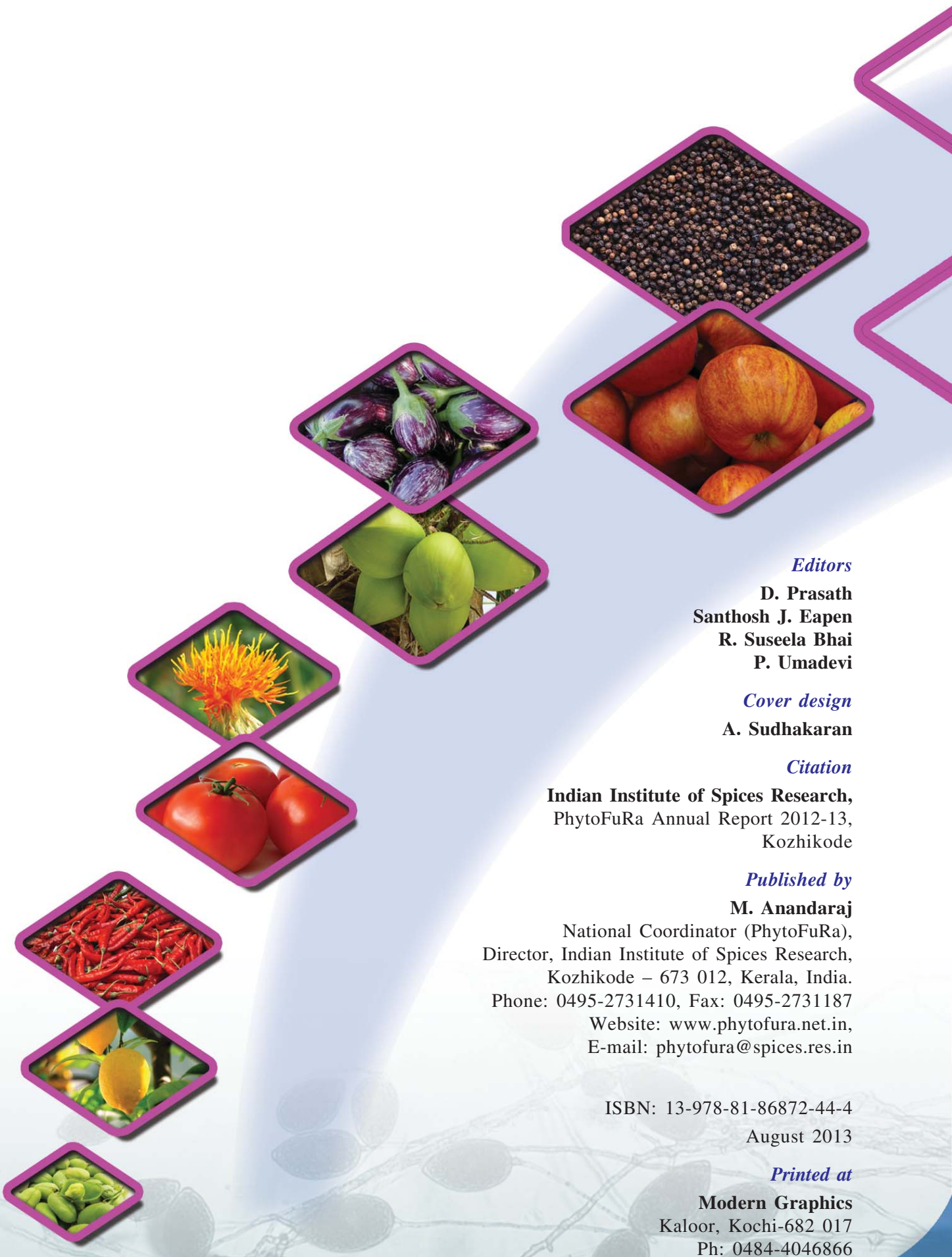

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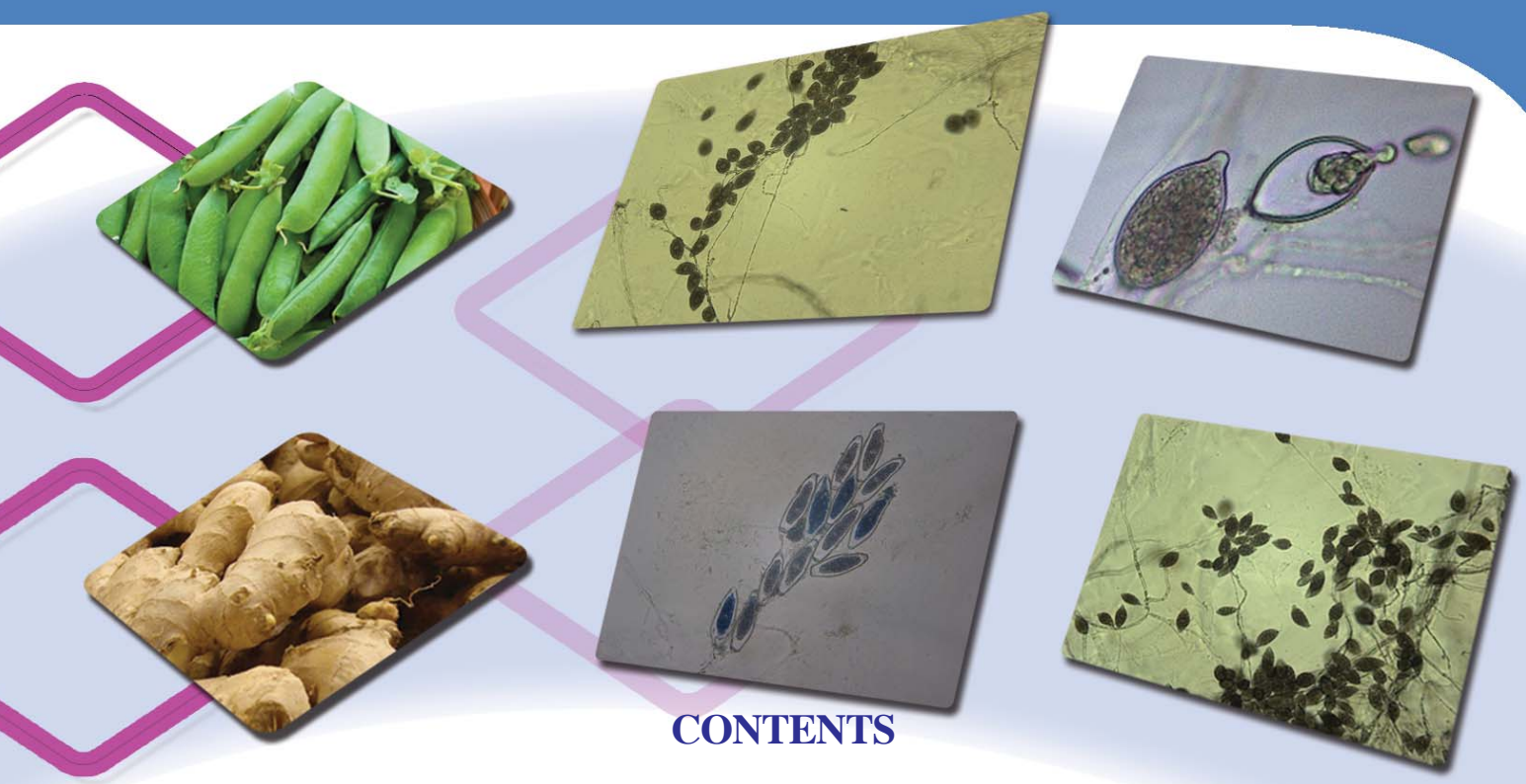

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PREFACE

The Outreach project on *Phytophthora, Fusarium* and *Ralstonia* diseases of Horticultural and Field Crops (PhytoFuRA), launched during 2008-09 is one of the major research initiatives of Indian Council of Agricultural Research, New Delhi to deal with the three major wilt pathogens of horticultural and field crops. The core objective of the project was to study the diversity of the these three pathogens and their interaction with the respective host plants and the environment in order to develop reliable detection techniques and management strategies.

I have great pleasure in presenting the Annual Report 2012-13. The research under the ambit of this project was done in six thematic areas *viz*., diversity, diagnostics, epidemiology, genomics and bioinformatics, host resistance and disease management.

Incidence and distribution of *Phytophthora* diseases of various crops were studied and 155 *Phytophthora* isolates were collected during the year. Genetic diversity and fingerprinting of *Phytophthora* isolates using different molecular markers suggested that *Phytophthora* isolates are at a rapid pace of evolution with high level of diversity. New *P. colocasia* isolates were collected and sporulation techniques were developed. *Phytophthora* resistant or tolerant lines were identified in apple, citrus and coconut through screening. Sequence data analysis of the interactive transcriptome of *Piper-Phytopthora* revealed involvement of genes *viz*., poly- galacturonases, pectin lyase and glycosyl hydrolases and other hydrolases of *Phytophthora*. Two QTLs located on chromosomes IX and X of diploid mapping population of *Solanum spegazzinii* x *S. chacoense* were identified. Four rhamnolipid congeners were identified from biosurfactant compounds obtained from *Pseudomonas aeruginosa* for management of *Phytophthora* disease in potato. Integrated disease management strategies were developed for control of collar rot of apple.

Variants of *Fusarium udum* and races of *F. oxysporum* f.sp. *ciceri* were analysed and their distribution in different states of India was documented. Promising *Fusarium* tolerant lines were identified in chickpea, pigeonpea, and tomato. Multilocation trials of *Trichoderma* isolates were found to be effective in reducing various diseases caused by *Fusarium*. Mining the microsatellite dynamics in whole genome of *F. oxysporum* f. sp. *lycopersici* was completed. The combined application of rhizospheric and endophytic fungal antagonists along with fungicide application significantly increased the bunch weight and suppressed the *Fusarium* wilt disease in banana.

During the year, 115 *R. solanacearum* isolates were collected from bacterial wilt affected target crops. Multi locus sequence typing (MLST) wad adopted as a tool for analyzing the genetic diversity existing among *R. solanacearum* isolates from solanaceous hosts. The results indicated that even though Indian strains look clearly original, they deserve further investigation. In case of tomato, cultivar *Arka Samrat* was found to be suitable for including in integrated bacterial wilt disease management. The biocontrol agents *P. fluorescens* and *Bacillus subtilis* for the control of bacterial wilt in tomato was studied under field condition with increased yield.

Multilocation testing of efficient *Trichoderma* strains against *Phytophthora* and *Fusarium* revealed some isolates were efficient in disease suppression, irrespective of the location or host plant indicating adaptation of the isolates to various niches.

The research progress and fund utilization was closely monitored through the custom made portal http://www.phytofura.net.in. The portal facilitated better and faster interaction and sharing of resources among the investigators and research fellows.

I consider it a privilege to place on record the encouragement given by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR. But for the strong support and guidance we received from Dr. N.K. Krishnakumar, Deputy Director General (Horticulture) and Dr. Umesh Srivastava, former ADG (Hort.II) and Dr S.K. Malhothra, the present ADG (Hort.II), we would not have made these achievements. I appreciate the efforts and zeal shown by all the investigators of this project in executing the programmes. The financial support for the project received from ICAR is gratefully acknowledged. I also appreciate the editors for having compiled and brought out this Annual Report.

15.08.2013, Kozhikode

(M. Anandaraj)

EXECUTIVE SUMMARY

Phytophthora

DIVERSITY

Incidence and distribution of *Phytophthora* diseases of various crops were studied and *Phytophthora* was isolated from the samples collected and maintained. During the period, 155 new *Phytophthora* isolates were collected, which includes 100 *P. infestans*, 14 *Phytophthora* isolates from apple, 41 isolates from citrus and *Phytophthora* spp. isolates (18 isolates of *P. nicotianae*, six isolates of *P. palmivora*, three isolates of *P. boehmeriae* and one isolate of *P. citrophthora*). Representative isolates from coconut, cocoa and citrus were deposited in the National Repository of *Phytophthora* at IISR Kozhikode, Kerala. A cryopreservation protocol was standardized for *P. infestans* and 700 isolates were maintained under liquid nitrogen at CPRI Shimla. Surveys conducted in apple growing areas *viz.,* Chamba, Sirmour and Kinnaur for collar rot disease (*P. cactorum*) showed that the disease incidence varied between 2.6-18.8%, 1.2-24.6%, and 0.4-8.30%, respectively. Maximum incidence of this disease was observed at Jattota and Baragarh of Chamba, Leonana in Sirmour and Lippa in Kinnaur districts of Himachal Pradesh.

The colony of *P. nicotianae* isolates showed dense cottony mycelium with no specific pattern of growth whereas *P. palmivora* isolates mostly produced a stellate striated pattern on V8 agar media. *P. boehmeriae* isolates showed petalloid pattern with irregular margin on V8 agar. *P. citrophthora* isolate showed colony with stellate pattern with irregular margin on V8 agar while floral/ pettaloid pattern was observed on PDA.

A multi-gene approach was attempted for the characterization of *Phytophthora* isolates from black pepper. This revealed distinct diversity among the isolates, there by classifying them into two groups.

Variability of selected *Phytophthora* isolates of cocoa and coconut was evaluated at molecular level by RAPD markers. All the 12 selected primers distinguished the different species of coconut and cocoa *Phytophthora*. Although the patterns within *P. palmivora* infecting cocoa were usually quite similar, there were intra specific variations with some of the primers. The most virulent *P. palmivora* isolate, AP/CA-334 and KL/CA-216 formed distinct clusters under two separate sub-clusters. Thus RAPD analysis revealed inter and intra specific diversity in *Phytophthora* spp./*P. palmivora* isolates.

The mitochondrial haplotyping of Indian isolates revealed that *P. infestans* population is composed of Ia haplotype.

Genetic diversity of *P. colocasiae* populations from different taro growing regions was studied using AFLP marker. The results showed the presence of high levels of genetic diversity and isolates were clustered together irrespective of their geographical origin.

For the first time, *P. colocasiae* isolates obtained from fine spatial scale (multiple leaf blight lesion on the single taro leaf) were analyzed for the presence of phenotypic and molecular diversity. Phenotypic characters revealed no variation. RAPD and ITS sequencing and analysis revealed considerable variation in the ITS1 region of all the isolates examined. These variations reinforce that the population of *P. colocasiae* are highly heterogeneous and are continuously evolving.

DIAGNOSTICS

New methods for detection of *P. infestans* in host tissues using real time PCR and a SCAR marker based on RAPD sequence were developed.

Real-time PCR assay for detection of *P. colocasiae* and *P. nicotianae* was developed. This assay was also used for the quantification of *P. colocasia* infected propagules.

Species specific primers were designed and successfully used for detecting *P. colocasiae* and *P. palmivora.*

A diagnostic ITS-RFLP profile based identification has been developed for the seven *Phytophthora* spp. *(P. capsici, P. nicotianae*, *P. palmivora*, *P. citrophthora, P. boehmeriae, P. insolita* and *P. lacustris*) isolated from black pepper and citrus.

HOST-PLANT RESISTANCE

Phytophthora resistant or tolerant lines were identified in apple, citrus and coconut through screening (Table 1).

Table 1. *Phytophthora* resistant/tolerant lines

Crop	Promising lines
Coconut	Accession, Malayan Green Dwarf (MGD) was identified as tolerant to *Phytophthora* infection
Apple	*Malus floribunda* showed maximum tolerance to the disease as minimum mortality to the extent of 12.8 and 10.2% under pot and nursery conditions, respectively
Citrus	Rough lemon 325 was found moderately tolerant

EPIDEMIOLOGY

The epidemiological studies of *P. cactorum* causing collar rot disease of apple showed that under nursery condition (sick plot), disease appeared in the first week of April and increased with the maximum soil temperature ranging between 21.1-25.9 and 20.4-27.2°C (up to 10 cm depth) during April to July and reached its maximum in the second week of August with the prevalence of 65.7-73.6 per cent soil moisture (up to 10 cm soil depth). Under orchard conditions, disease appeared on 21 March and increased in a similar manner as nursery conditions.

The studies on rhizosphere microflora of apple trees showed that *Phytophthora* containing soil samples had low population of *Trichoderma*, *Penicillium* and bacterial species whereas, species of *Aspergillus* and *Pythium* were present in higher level and reverse for non *Phytophthora* containing soil**.**

DISEASE MANAGEMENT

Four rhamnolipid congeners were identified from biosurfactant compound obtained from *Pseudomonas aeruginosa*. A rhamnolipid based formulation was developed and tested on detached leaves.

The biocontrol agents TV5 (*Trichoderma viride* 5), TH15 (*T. harzianum* 15) and *Bacillus* sp 11 were found to be compatible with fungicide, metalaxyl MZ and provided enhanced (>98%) disease control when applied in combination.

Among the new fungicides evaluated against *P. cactorum,* curzate was found to be highly effective in controlling the disease both under nursery and orchard conditions.

Fourteen fungal and sixteen bacterial antagonists were isolated from the soil samples of two apple growing districts (Sirmour and Chamba). Evaluation under *in vitro* and pot conditions indicated that *Trichoderma harzianum*-5 and *Bacillus* sp-4 were highly effective (PDC>75% when applied 14 days prior to infection).

A new bio-control agent (*Burkholderia cepacia*-isolate PB2) was isolated from citrus regime and was found effective against *Phytophthora* sp. with maximum inhibition percentage (45.94%).

Among 15 isolates of *Trichoderma* collected from different geographical locations, the PhytoFuRa10 was found to be highly effective against *P. capsici* infection in black pepper with less than 10% disease incidence followed by PhytoFuRa 8, PhytoFuRa 11, and PhytoFuRa 13. In the case of citrus, the isolates PhytoFuRa 6 followed by PhytoFuRa 5 and PhytoFuRa 11 showed maximum root rot reduction in *in vivo* tests.

Integrated disease management strategies were developed for collar rot of apple which included soil drenching of *Trichoderma viride* 5 / *T. harzianum* 15 + *Bacillus* sp. 11 / *Enterobacter aerogenes* -2 or amendments (mustard cakes + vitex leaf) or BCAs + mustard cake along with metalaxyl MZ (0.3%). Almost complete control (>98%) of disease was obtained when applied prior to planting of seedlings under nursery conditions.

In apple, approach grafting combined with application of cow urine decoction of *Vitex* leaves and *Melia azedarach* seeds in the month of March and metalaxyl MZ (0.3%) during April and August was most effective and recorded maximum increase in shoot length.

HOST-PATHOGEN INTERACTION

Sequences with homology to lectin-like receptor kinase a potential host target for RxLR type of effectors from *Phytophthora* was discovered.

Sequences of polygalacturonase inhibitor protein, a defense gene, potential to be used in the resistance breeding was identified in *Piper* sp.

Members of transcriptional factor gene families *viz*., *NAC, BHLH, MYB, MYC, BZIP, WRKY* etc.

important in the regulation of genes under biotic and abiotic stress were identified.

A number of SNPs were discovered in specific genes *viz*., defensin, thaumatin-like protein, cysteine proteinase inhibitor, polygalacturonase inhibitor protein, lectin-like receptor kinase and other genes including transcription factore that are involved in *Piper colubrinum – Phytophthora capsici* interaction.

Gene expression studies in *P. colubrinum* challenge inoculated with *Phytophthora* revealed highest level of expression in case of polygalacturonase inhibitor protein, indicating its importance in disease resistance compared to other defense genes and transcription factors.

Sequence data analysis of the interactive transcriptome reveled involvement of genes *viz*., poly- galacturonases, pectin lyase and glycosyl hydrolases and other hydrolases of *Phytophthora* in the interactions with *Piper*.

GENOMICS AND BIO-INFORMATICS

The NBS analogs of *P. nigrum* were identified to be non-TIR-NBS-LRR subfamily with highly conserved tryptophan (W) as the last residue of kinase-2 domain. *Piper* RGCs shows significant sequence similarity to the Kinase-1a, Kinase-2 and hydrophobic GLPL motifs of the NBS domain of the known genes. Phylogenetic tree shows that *P. nigrum* NBS analogs are of different origin forming 2 separate clusters.

Bioassay and qPCR analysis confirmed the resistant nature of siRNA and amiRNA transgenic lines against late blight in potato.

Out of 64, 38 *Solanum* species showed presence of 53 different *R* genes conferring late blight resistance in potato. Identified 15 clones possessing R1 and R3a genes in a single host back ground through MAS.

Identified two QTLs located on chrosomes IX and X of diploid mapping population of *Solanum spegazzinii* x *S. chacoense*.

Sequenced whole genome of A2 mating type.

Fusarium

DIVERSITY

Variants of *F. udum* and races of *F. oxysporum f.sp. ciceri* were analysed and their distribution in different states of India was documented. Diversity in *F. udum* was very high in Uttar Pradesh with seven variants followed by Karnataka, Maharashtra and Bihar, Madhya Pradesh, Andhra Pradesh, Rajasthan while other states had 1-2 variants. Diversity in *F. oxysporum* f. sp. *ciceri* was also very high in Uttar Pradesh and Rajasthan (five races) followed by Madhya Pradesh, Maharashtra and Haryana while other states showed the presence of 1-2 races only.

Genetic diversity in *Fusarium* isolates were analysed using molecular markers *viz*., RAPD (*F. udum and F. oxysporum* f. sp. *ciceri*), SSR (*F. udum*), ISSR (*F. solani* and *F. oxysporum*), SSR (*F. oxysporum* f. sp. *lycopersici),* IGS-RFLP (*F. oxysporum* f. sp. *ciceri*), *TEF*-1α gene (*F. oxysporum* f. sp. *psidii*, *F. oxysporum* f.sp. *carthami, F. oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *ciceri, F. solani* and *F. udum*), ITS (*F. udum, F. oxysporum* f. sp. *psidii, F. oxysporum* f. sp. *lycopersici*, *F. solani, F. oxysporum* f. sp. *ciceri* and *F. udum),* REP-PCR, ERIC-PCR and BOX-PCR (*F. oxysporum* f. sp. *psidii*), SRAP (*F. oxysporum* f. sp. *ciceri*), chitin synthase (virulent group of *Fusarium* spp.), β-tubulin (*F. oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *ciceri, F. solani and F. udum*) and mitochondrial markers (*F. oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *ciceri, F. solani* and *F. udum).*

DIAGNOSTICS

Species specific marker was developed for identification of *F. oxysporum* f. sp. *psidii* isolates. Also, nested colony PCR assays were developed for early and rapid detection of *F. oxysporum* f. sp. *psidii* isolates.

A PCR technique for identification of *Fusarium* spp. in soil samples using nested PCR using ITS primers were developed which can detect the pathogen even at 10^{-7} and 10^{-8} dilutions.

Highly sensitive β-tubulin gene and IGS region based molecular markers were developed for detection of *F. oxysporum* f. sp. *ciceri* using real time PCR.

HOST-PLANT RESISTANCE

Promising *Fusarium* resistant/tolerant lines were identified in chickpea (29), pigeonpea (13), and tomato (7).

Polymorphic EST-SSR markers specific to wild species *viz., C. oxyacantha, C. palaestinus, C. lanatus, C. creticus, C. glaucus* and *C. turkistanicus,* respectively have been identified. One hundred and forty one genomic SSRs screened for identifying the polymorphic markers.

Differential expression patterns of the defense related genes in response to *F. oxysporum* f. sp. *ciceri* infection in resistant and susceptible varieties of chickpea were analysed and chalcone synthase gene was found to be down regulated in resistant variety after 24 h of inoculation and upregulated after 96 h of *F. oxysporum* f. sp. *ciceri* inoculation and chemical inducers like salicylic and jasmonic acid.

DISEASE MANAGEMENT

A number of fungal isolates *viz*., *Trichoderma harzianum*, *T. viride* (Kanpur), *T. virens* (Bengaluru), *T. longibrachyatum,* non-pathogenic *Fusarium* strains were isolated, screened and evaluated for control of various diseases caused by *Fusarium*. Two non-pathogenic *Fusarium* strain *viz*., Fu4 and Fu showed inhibitory potential against *F. udum* and *F. oxysporum* f.sp. *ciceri*.

The combined application of rhizospheric and endophytic fungal antagonists along with or without fungicide application significantly increased the bunch weight and suppressed the *Fusarium* wilt disease in banana. The biopriming of banana plants with the combined application of *Pseudomonas putida* + *Alpinia/Hibiscus* sp./Zimmu, *Bacillus* sp. + Zimmu combinations resulted in complete control (100% reduction) of the disease under pot culture condition.

The quantification of *Trichoderma* spp. population of both endophytic and rhizhospheric origin in the rhizosphere soil and shelf life of *Trichoderma* spp. in liquid formulation were standardized for management of *Fusarium* wilt in banana.

Botanical extracts of *Datura* and garlic were able to reduce disease incidence in tomato at significant level.

A combination of seed treatment with *T. harzianum*, (Pusa 5SD) *P. fluorescens* (Pf 80) and vitavax powder provided significantly higher seed germination, grain yield and the lowest wilt incidence in chick pea under field conditions. Seed treatment with carbendazim was most effective in reducing seedling wilt in pigeonpea.

GENOMICS AND BIO-INFORMATICS

Mining the microsatellite dynamics in whole genome of *F. oxysporum* f. sp. *lycopersici* was completed.

Ralstonia

DIVERSITY

One hundred and fifteen *R. solanacearum* isolates (tomato, brinjal, chilli, capsicum, potato and ginger) were collected from different states of India including Uttarakhand, Meghalaya, Jammu and Kashmir, Himachal Pradesh, Jharkhand, Orissa, West Bengal and Kerala.

Among 95 potato isolates collected from Uttarakhand and Meghalaya, 32 belong to biovar 2, 19 to biovar 3 and 44 to biovar 2T. The race profiling and phylotyping of other solanaceous isolates of *R. solanacearum* proved that they belong to race 1 and phylotype 1 and biovar 3 and 4. Virulence of 196 *R. solanacearum* isolates on brinjal was evaluated and the results indicated that the isolates vary in virulence.

Multi locus sequence typing (MLST) wad adopted as a tool for analyzing the genetic diversity of existing *R. solanacearum* isolates from solanaceous hosts, the results indicate that even though Indian strains look clearly original, and deserve further investigations.

The sequence analysis of *hrp* gene segment was employed to characterize *Ralstonia* isolates obtained from wilted tomato, chilli, and eggplant in different locations of Karnataka, Kerala, Delhi and Goa. The comparative sequence analysis reveals that the isolates from India differ with already reported *R. solanacearum* isolates and other *Ralstonia* species.

Three methods were tested for screening tomato, brinjal and chilli entries for bacterial wilt resistance *viz*., soil drenching, leaf clipping and axil puncturing. The soil drenching method was found to be the best, which gave 98% wilt incidence.

HOST RESISTANCE

In order to find the biochemical mechanism involved in the bacterial wilt resistance of *Curcuma amada,* the essential oil was extracted from the dried rhizomes of *C. amada* which exhibited maximum antimicrobial activity on *R. solanacearum*. Beta-myrcene and beta-pinene were the major components in essential oil.

To decipher the mechanism of bacterial wilt resistance in *C. amada,* the NBS region was amplified and sequenced. The cluster analysis showed that the NBS analogs of *C. amada* (531 bp) and *Z. officinale* (534 bp) belonged to non-TIR-NBS-LRR subfamily with highly conserved tryptophan as the last residue of kinase-2 domain.

The functionally annotated subtracted transcriptome on host pathogen interaction in ginger revealed defense/stress related genes like glutathione-s-transferase, leucine rich protein and various enzymes involved in anti-oxidant defense.

In tomato expression of defense related *genes PR*-1a and *GluA* (salicylic acid pathway), *Pin2* and *LoxA* (jasmonic acid pathway) and *PR*-1b and osmotin like (ethylene pathway) in resistant (Hawaii 9796) and susceptible (Pusa Ruby) cultivars against the *R. solanacearum* strain UTT-24 were assessed.

DISEASE MANAGEMENT

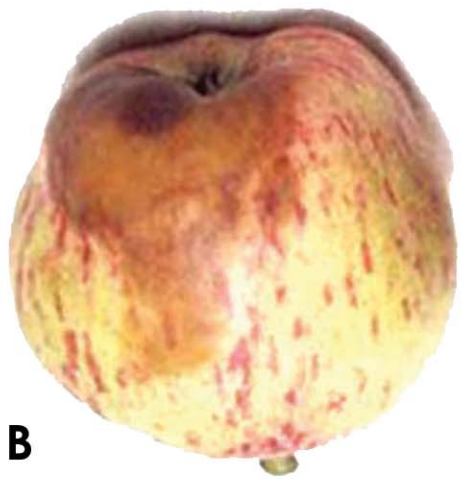
Different strategies were employed to control the bacterial wilt disease. Phages, which specifically infect *R. solanacearum*, were isolated from ginger rhizosphere soil using *R. solanacearum* as the host. Also, 150 bacteria were isolated from the apoplastic fluid collected from the pseudostem and leaves of ginger. *Trichoderma* species alone or in combination with *P. fluorescens* was found effective in increasing plant growth parameters and rhizome development in ginger.

In tomato, cultivar Arka Samrat was found to be suitable for including in the integrated bacterial wilt disease management. In the field condition, Arka Samrat recorded reduced wilt incidence with application of bleaching powder @ 15 kg/ha and *P. fluorescens*.

The biocontrol agents, *P. fluorescens* and *Bacillus subtilis* in the control of bacterial wilt in tomato was determined under field condition with increased yield.

PhytoFuRa

Phytophthora



Phytophthora disease symptoms on A. Potato, B. Apple, C. Black pepper, D. Colocasia

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a. *PHYTOPHTHORA* DIVERSITY

A multi-gene approach for the characterization of *Phytophthora* isolates from black pepper

ITS_RFLP profiling of *P. capsici* isolates from black pepper, grouped the isolates into group I and II. The sequence analysis of ITS region showed that the isolates belonging to group I had close resemblance to *P. capsici* isolates deposited in NCBI, whereas those of group II were having resemblance to both *P. tropicalis* and *P. capsici.*

A multigene approach was attempted for further characterizing these isolates by taking two representative isolates from each group. For this, nine loci were selected *viz.,* 28S ribosomal DNA, 60S ribosomal protein L10, β-tubulin, elongation factor 1α, enolase, heat shock protein 90, *Tig*A gene fusion protein (Blair *et al.,* 2008)*;* mitochondrial genome region between gene *Cox2* and gene *Cox1* and Ras-related protein (*Ypt1*) gene (Schena and Cooke, 2006). All the loci were amplified using corresponding primers. Phylogenetic trees were reconstructed for each marker using neighbor-joining with a Kimura two parameter nucleotide substitution model (Fig. 1). This showed high diversity among the members of group II from both *P. capsici* and *P. tropicalis.*

The cross infectivity studies of these isolates on *Capsicum* showed that all are pathogenic on *Capsicum* with the members of group II having high virulence on it. The isolates belonging to group II formed chlamydospores on carrot agar media whereas those of group I did not form any chlamydospores.

b. HOST PATHOGEN INTERACTION

Transcriptome analysis on (*Piper colubrinum – Phytophthora capsici*) interaction

The transcriptome data analysis derived from *Piper nigrum* and *P. colubrinum* leaves challenged with *P. capsici* revealed identification of many defense related genes differentially expressed in these two different species of *Piper.* Sequences with homology to lectin-like receptor kinase, antimicrobial small peptides such as defensins, polygalacturonase inhibitor protein (PGIP), defense-associated transcription factors belonging to the family of *NAC, BHLH, MYB, MYC, BZIP* etc. were recovered in this analysis. Analysis of allelic variants in selected genes (Table 2) revealed maximum SNPs in MAP kinase gene compared to other genes like PAL, polyphenol oxidase, etc.

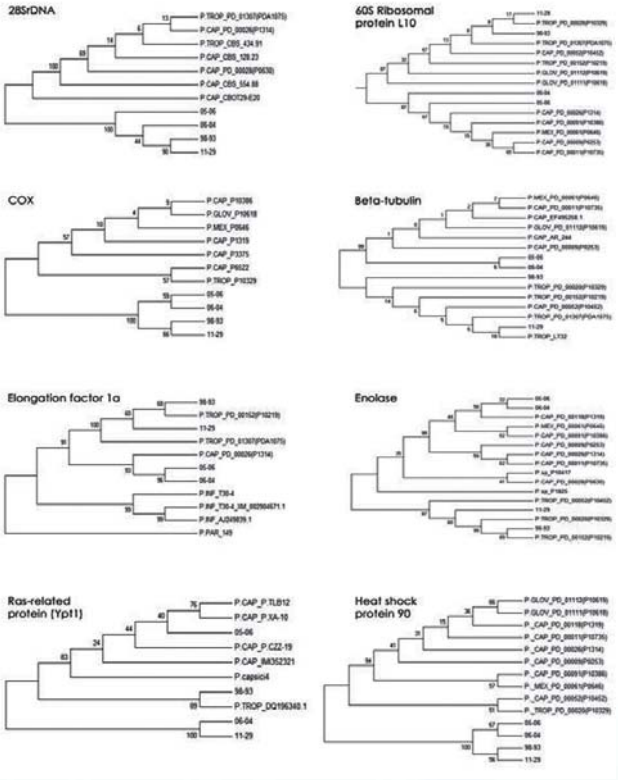

Fig. 1. Phylogenetic trees based on eight genes in *Phytophthora capsici* isolates infecting black pepper

Molecular analyses of the expression of five defense-associated genes were carried out using real-time quantitative reverse transcription-polymerase chain reaction (qPCR) assays. The expression profiles of different defense related genes differed significantly in the studies

Table 2. SNPs discovered in specific genes involved in *Piper colubrinum – Phytophthora* interaction

Gene identity	No. of bases	No. of SNPs
Defensin	212	1
Thaumatin-like protein	577	2
Cysteine proteinase inhibitor	1160	2
Polyphenol oxidase	1938	2
Polygalacturonase inhibitor protein	294	0
Phenylalanine ammonia lyase	386	1
BHLH transcriptional factor	1423	2
MYB transcription factor	1286	7
MYC transcription factor	1756	5
MAP kinase	3069	11
WRKY transcription factor	2042	6
Class I chitinase	1042	5
Lectin-like receptor kinase	157	0

undertaken in *P. colubrinum* leaves challenged with *P. capsici*. The fold expression was very high in case of polygalacturonase inhibitor protein, followed by polyphenol oxidase, thaumatin-like protein, defensin and cysteine proteinase inhibitor gene (Fig. 2).

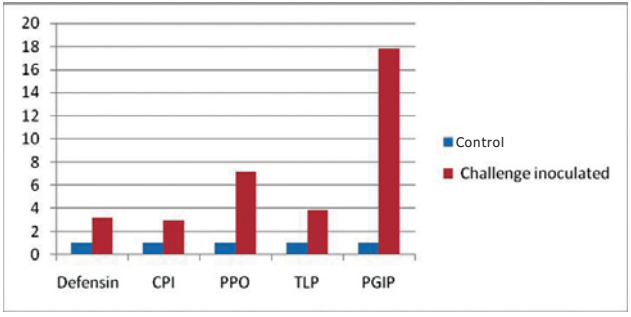

Fig. 2. Relative expression of defense related gene in *P. colubrinum* challenge inoculated with *Phytophthora*. CPI- Cysteine proteinase inhibitor, PPO-Polyphenol oxidase, TLP-Thaumatin-like protein and PGIP-Polygalacturonase inhibitor protein

c. HOST RESISTANCE

ISSR profiling of progenies of Panniyur 1 x Subhakara cross

Panniyur 1 and Subhakara (parents of mapping population) and their progenies were screened with 20 ISSR primers. Ten primers that showed polymorphism between parents were further used to profile 94 selected progenies of Panniyur1 x Subhakara (Fig. 3).

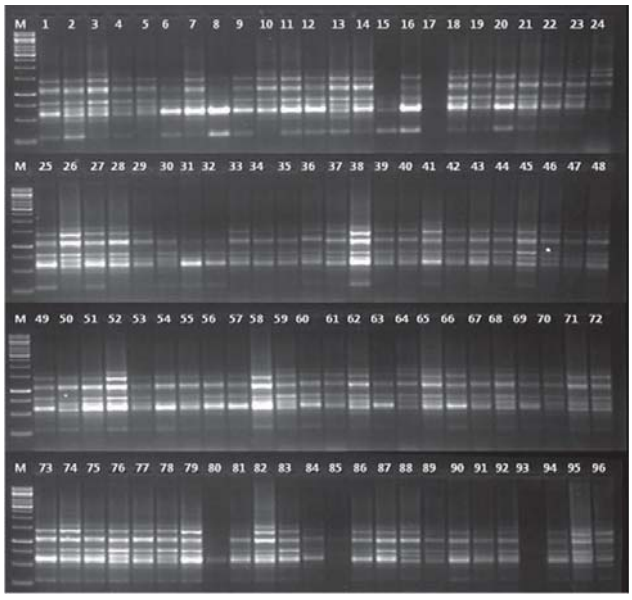

Fig. 3. ISSR profiling of black pepper mapping population of Panniyur 1 x Subhakara cross using the primer, UBC834a–$(AG)_8$CT

Amplification of resistance gene candidates

R gene candidates in the wild species *viz*., *P. colubrinum* (Acc. 392) and *P. ornatum* (Acc. 3362), two moderately resistant varieties (IISR Sakthi and P24-O-4) and two susceptible varieties (Subhakara and Sreekara) were amplified using the primer pair, P6F 5'-GGACCTGGTGGGGTTGGGAAGACAAC-3' and P6R 5'-CAACGCTAGTGGCAATCC-3' (Ohimori *et al*. 1998) (Fig. 4). Sequence analysis of *Piper* RGAs showed the presence of conserved domains *viz*., kinase-1a, kinase-2 and hydrophobic motif, provided evidence that the sequences belong to the NBS-LRR class gene family. The presence of tryptophan as the last residue of kinase-2 motif further qualified them to be in the non-TIR NBS-LRR subfamily of resistance genes. *P. nigrum* RGAs formed two groups with *P. colubrinum* resistance gene analogs (Fig. 5). *Piper* RGA sequences derived using the R-gene specific degenerate primers have been deposited in the GenBank database under the accession numbers: JX416288-JX416291 and JX898031-JX898033.

d. DISEASE MANAGEMENT

Molecular identification of endophytic fungal isolates

The ITS region of two endophytic fungi isolates of (BPEF-11 and BPEF-83) were amplified using the universal primer ITS4 and ITS5 which yielded 600 bp product. PCR products were purified and

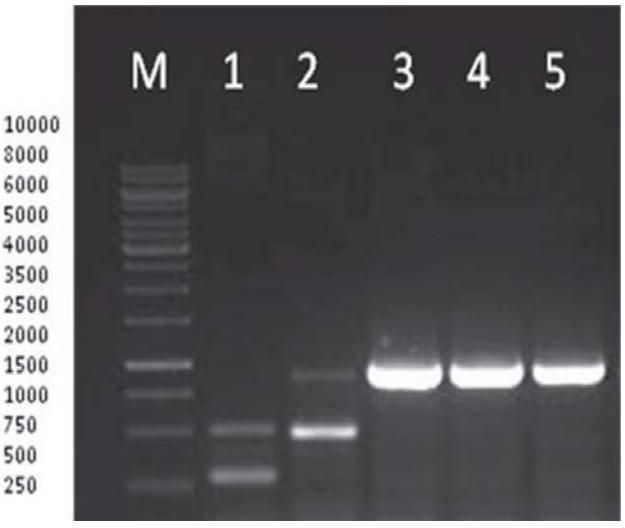

Fig. 4. Amplification of R gene analogues in *Phytophthora* resistant and susceptible *Piper* collections using primer combinations, P6 F and R. M-1Kb, 1- *P. colubrinum*, 2 - IISR Sakthi, 3 - P24-O-4, 4 – Sreekara and 5- Subhakara

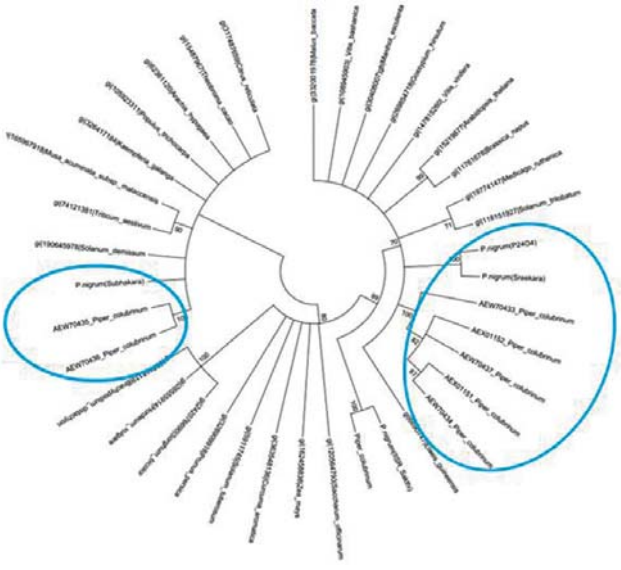

Fig. 5. Average distance tree showing relationship of *P. nigrum and P. colubrinum* NBS analogs with the characterized NBS domain of R genes from other plant species

sequenced. Sequence similarity search indicated that the products contain ITS1, 5.8 S ribosomal subunit and ITS2 regions. The sequence from the isolate BPEF-11 showed maximum identity (99%) with *Diaporthe* sp. while the isolate BPEF-83 had maximum identity (97%) with *Phomopsis* sp.

Studies on gnotobiotic nature of endophytic fungi

Ripened seeds of black pepper (Panniyur 1) were collected; surface sterilized and placed in water agar tubes for germination. Total thousand seeds were kept for *in vitro* germination. Sections made out of these *in vitro* raised plants were plated separately on malt extract agar (MEA) to which 0.1% stock antibiotic solution was added (stock: 0.02 g each tetracycline, streptomycin and penicillin in 10 ml sterile distilled water, filter sterilized; from this 1 ml was added per liter of media) and incubated at room temperature. Fungal growth was not observed in 81% of the seedlings whereas 5% of the seedlings showed fungal growth in all the three tissues (stem, root and leaf). Another set of sections were surface-sterilized by dipping in 0.5% sodium hypochlorite for 2 min, 70% ethanol for 2 min and rinsed in sterile distilled water followed by drying on sterile filter paper (Arnold *et al.* 2001). The edges of each sampled tissue were cut off and discarded and subsamples of the remaining tissue (approx. 2 mm x 3 mm in size) were plated as described above. Sterilized tissue segments were pressed on to the surface of MEA medium to check the efficacy of surface sterilization procedure. Out of the seedlings analyzed 96% were free from endophytic association and only 4% of tissues showed fungal growth in sterility check. The study clearly revealed that the endophytic fungi isolated from black pepper are not gnotobiotics.

Multi location testing of *Trichoderma* isolates

Pot culture experiments were conducted with 15 geographically different isolates of *Trichoderma* in black pepper varieties Panchami and Sreekara. Uniform sized black pepper cuttings were planted in non sterilized potting mixture (pH 6.5) having a microbial load of 10^7, 10^4 and 10^3 for bacteria, fungi and *Trichoderma*, respectively. At the time of planting, first application of *Trichoderma* (10^8 spores/ml) was done. The plants were challenge inoculated with *P. capsici* (05-06) and plant growth, disease symptoms and *Trichoderma* population were monitored at monthly intervals (Table 3). The fungal population did not show much difference where as there was a substantial increase in *Trichoderma* population. PhytoFuRa 10 showed minimum disease incidence (8.17%) followed by PhytoFuRa 3 (10.68%). PhytoFuRa 4, 5, 6 and 7 showed more than 80% disease incidence in Panchami. The treatment with PhytoFuRa 13 showed maximum plant height. In the case of variety Sreekara, treatment PhytoFuRa 10 showed less disease incidence followed by PhytoFuRa 11, 12 and 13 (less than 20% disease incidence). The treatment with PhytoFuRa 2 showed maximum plant height.

Table 3. Disease incidence and soil microbial count in black pepper plants treated with different isolates of *Trichoderma*

Treatment	Panchami				Sreekara			
	Trichoderma population	Total culturable fungi	Total culturable bacteria	Disease incidence (%)	*Trichoderma* population	Total culturable fungi	Total culturable bacteria	Disease incidence (%)
PhytoFuRa 1	$3x10^7$	$4x10^5$	$6x10^6$	40.27	$2x10^7$	$2x10^5$	$9x10^6$	22.91
PhytoFuRa 2	$6x10^7$	$1x10^5$	$3x10^6$	55.27	$1x10^8$	$4x10^5$	$8x10^6$	37.63
PhytoFuRa 3	$5x10^7$	$1x10^5$	$7x10^7$	10.68	$3x10^7$	$3x10^5$	$2x10^6$	22.91
PhytoFuRa 4	$3x10^5$	$2x10^5$	$9x10^6$	90.00	$2x10^6$	$8x10^3$	$5x10^7$	80.00
PhytoFuRa 5	$7x10^6$	$5x10^5$	$8x10^6$	90.00	$9x10^6$	$5x10^5$	$7x10^6$	70.00
PhytoFuRa 6	$7x10^7$	$3x10^5$	$6x10^7$	90.00	$2x10^7$	$7x10^5$	$7x10^5$	67.50
PhytoFuRa 7	$6x10^7$	$5x10^5$	$8x10^7$	80.00	$7x10^7$	$2x10^5$	$4x10^7$	57.50
PhytoFuRa 8	$8x10^7$	$2x10^5$	$2x10^6$	18.04	$1x10^7$	$2x10^5$	$2x10^6$	20.41
PhytoFuRa 9	$2x10^7$	$7x10^5$	$8x10^6$	60.13	$3x10^7$	$1x10^5$	$3x10^6$	60.00
PhytoFuRa 10	$6x10^7$	$6x10^5$	$3x10^6$	8.17	$7x10^7$	$5x10^3$	$4x10^5$	10.54
PhytoFuRa 11	$7x10^7$	$3x10^5$	$3x10^7$	30.41	$8x10^7$	$6x10^5$	$6x10^6$	12.21
PhytoFuRa 12	$8x10^6$	$4x10^5$	$8x10^7$	40.40	$6x10^6$	$3x10^5$	$8x10^7$	37.63
PhytoFuRa 13	$7x10^7$	$1x10^5$	$6x10^6$	25.54	$1x10^7$	$7x10^5$	$9x10^6$	13.04
PhytoFuRa 14	$3x10^7$	$2x10^5$	$4x10^6$	60.00	$2x10^7$	$8x10^5$	$8x10^6$	42.63
PhytoFuRa 15	$8x10^7$	$6x10^3$	$1x10^6$	25.54	$4x10^7$	$4x10^5$	$7x10^6$	35.13
Met-Mnz	Nil	$5x10^5$	$6x10^6$	55.13	Nil	$4x10^5$	$9x10^6$	37.77
Control	Nil	$8x10^5$	$7x10^7$	85.00	Nil	$2x10^5$	$5x10^6$	80.00

e. GENOMICS & BIOINFORMATICS

Genome sequencing and annotation

The complete genome of two *Phytophthora* isolates (05-06 and 98-93) infesting black pepper was sequenced using Illumina/Roche 454 platforms. The cross-platform sequence data was *de novo* assembled and annotated structurally and functionally to curate all possible gene by gene information.

Isolate 05-06 and 98-93 consist of a total genome size of 63.8Mb and 46.1Mb, respectively in hybrid assembly, obtained from 20.96 million high quality reads with average length of 101 bp generated using next generation sequencing method - Illumina-Solexa 1G Genetic Analyzer and Roche 454 GSFLX. Quality checks revealed that 99.54% HQ bases are present in 05-06 isolate while it is 99.71% in 98-93 isolate. *De novo* assembly resulted in 32044 and 9831 contigs for 05-06 and 98-93 with peak depth of 5 and 6, respectively. Structural annotation provided a total of 16356 genes, 38947 exons and 16356 conserved domains (CDS) for 05-06 isolate compared to 13068 genes, 33813 exons and 13068 CDS for 98-93 isolate. The number of predicted proteins was 7154 in 05-06 and 9344 in 98-93 isolate. SSR statistics was 1344 in 05-06 and 2496 in 98-93.

Whole genome alignment with the reference genome revealed that 05-06 is 95.35% similar while 98-93 is 87.90% similar to the reference genome. Variant annotation revealed that there are 113068 SNPs in 05-06 compared to 37839 SNPs in 98-93. About 2039 genes are conserved between 05-06 and 98-93 isolates. Genes unique to 98-93 are 6095 in numbers while 4034 genes are unique to 05-06. Functional annotation using BLAST search revealed the presence of various proteins important for the survival of *Phytophthora* sp. in host plants and virulence associated proteins crucial for its infection.

Conserved domain identification

Conserved domain search to identify the protein families present in exonic regions of whole genome sequences of the two different isolates of *Phytophthora* sp. infecting black pepper (05-06 and 98-93) has been carried out along with Blast2GO analysis.

Species and evolutionary tree estimation of sequenced *Phytophthora* isolates

The evolutionary history of the *Phytophthora* genomes sequenced was traced out using information from multilocus gene trees for four mitochondrial and 10 nuclear markers from six

closely related species of Clade 2 *viz., P. tropicalis*, *P. capsici*, *P. citricola*, *P. citrophthora*, *P. botryosa*, *P. meadii*, along with 05-06 and 98-93 IISR isolates. Our concatenation-based multispecies coalescent approach using Bayesian, maximum parsimony and maximum likelihood methods was able to estimate a moderately supported species tree showing a close relationship among 05-06 with *P. capsici* and 98-93 with *P. tropicalis*. The study clearly warrants a relook at the taxonomic identity of *Phytophthora* species infecting black pepper in India.

Development of *Piper* transcriptome database

Transcriptome data *of P. nigrum* and *P. colubrinum* plants challenged with *P. capsici* have been compiled and archived as a searchable database, *Phytophthora – Piper* TranscriptomeDB. This database provides access to the transcriptome sequences, number of genes, functional annotation, gene ontology annotations to research community (Fig. 6).

Fig. 6. A snapshot of *Phytophthora – Piper* transcriptome database

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE, KASARAGOD

Principal Investigator:

Dr. R. Chandramohanan (upto 31 Oct 2012)
Dr. Vinayaka Hegde (from 1 Nov 2012)

a. *PHYTOPHTHORA* DIVERSITY

Molecular characterization of *Phytophthora* isolates

Cultural, morphological and pathogenic characterisation of *Phytophthora* isolates of coconut and cocoa collected from different locations in four Southern states of India revealed distinct variations not only between species but also among the isolates within a species, especially *P. palmivora.* The diversities were further confirmed by randomly amplified polymorphic DNA (RAPD) analysis of selected isolates of *Phytophthora* spp. Based on initial screening of RAPD primers, 12 primers were selected for analysis of genetic variation on 24 isolates of *P. palmivora,* four isolates of *P. nicotianae* and one isolate each of *P. meadii* and *P. capsici* of coconut. All four species of *Phytophthora* could be distinguished with the 12 selected primers. Thirty *Phytophthora* isolates falls into two distinct main clusters. There were two subclusters under the first main cluster (Fig. 7). *P. palmivora* isolates formed one group whereas the single isolate of *P. meadii* formed a separate subcluster. The second main cluster consisted of single isolate of *P. capsici* in one subcluster and four isolates of *P. nicotianae* which formed two distinct subclusters. The patterns within *P. palmivora* were usually quite similar with some intra-specific variations.

In a similar study, 25 isolates of *P. palmivora* and one isolate each of *P. citrophthora*, *P. capsici*, *P. meadii* and *P. tropicalis* infecting cocoa were screened with the above 12 selected primers. Although the patterns within *P. palmivora* were usually quite similar with some intra specific variations with some of the primers, RAPD analysis separated the 29 *Phytophthora* isolates into two distinct main clusters (Fig. 8). There were three sub-clusters under one main cluster. The most virulent *P. palmivora* isolate, AP/CA-334 and KL/CA-216 formed distinct clusters under two separate sub-clusters. The analysis revealed the inter- and intra-specific diversity in *Phytophthora* spp. / *P. palmivora* isolates.

b. HOST RESISTANCE

Screening coconut and cocoa accessions against *Phytophthora* resistance

The rate of spread of infection (lesion size) caused by the six selected isolates of coconut *viz.,* KL/CO-8, KL/CO-1 and KL/CO-78 (*P. palmivora* isolates), KL/CO-16 (*P. meadii*), TN/CO-127 (*P. nicotianae*) and KA/CO-110 (*P. capsici*) were determined using the detached leaf technique (Fig. 9).

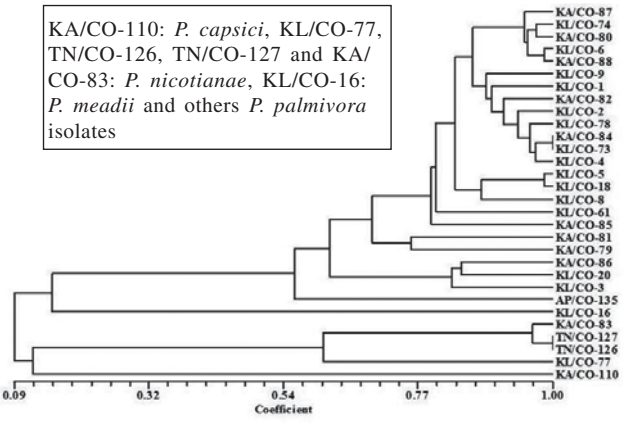

Fig. 7. UPGMA dendrogram of the relationships between isolates of *Phytophthora* species from coconut based on similarity (DICE) coefficients

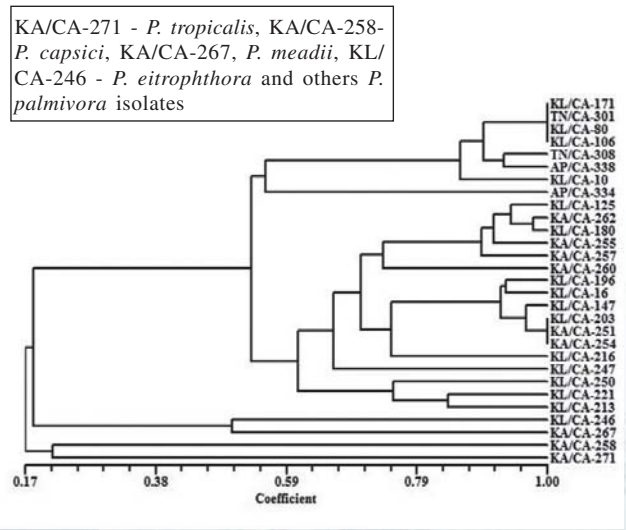

Fig. 8. UPGMA dendrogram of the relationships between isolates of *Phytophthora* species from cocoa based on similarity (DICE) coefficients

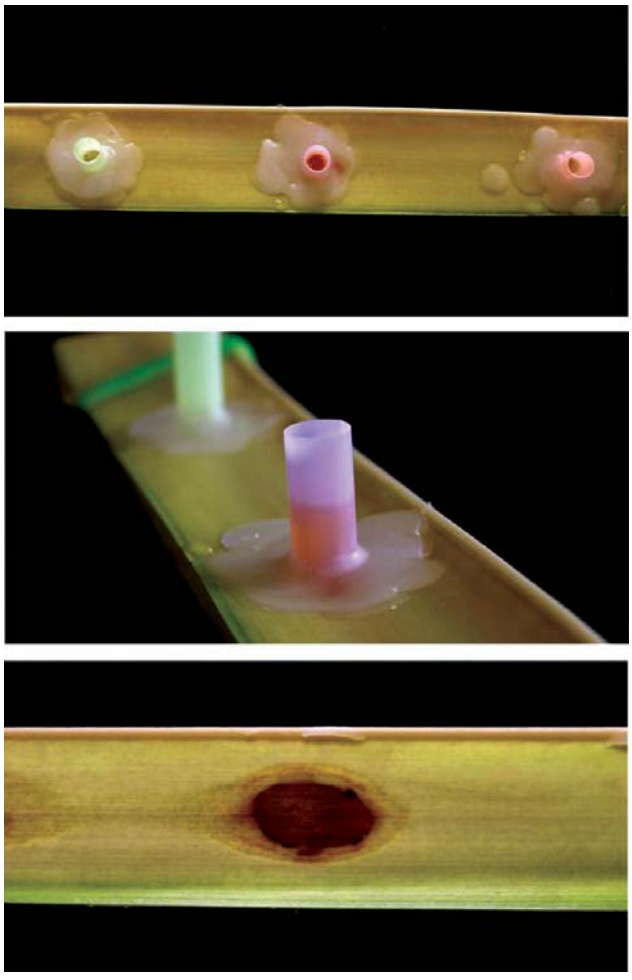

Fig. 9. A new detached leaflet inoculation technique in coconut

KA/CO-110: *P. capsici*, KL/CO-77, TN/CO-126, TN/CO-127 and KA/CO-83: *P. nicotianae*, KL/CO-16: *P. meadii* and others *P. palmivora* isolates

The susceptibility of 10 commonly cultivated accessions/hybrids *viz.,* WCT (West Coast Tall), COD (Chowghat Orange Dwarf), CGD (Chowghat Green Dwarf), DxT, TxD, LO (Laccadive Orange), MGD (Malayan Green Dwarf), Malayan Yellow Dwarf (MYD), GBGD (Ganga Bondam Green Dwarf) and Benaulim Green Round, to *Phytophthora* infection varied with the accessions and with the *Phytophthora* species when detached tender leaflets were used for inoculation. The infection index calculated from the size of the lesions recorded also revealed the susceptibility/resistance of coconut accessions to the four species of *Phytophthora* as well as variability within *P. palmivora*. In general, LO was found to be the highly susceptible accession to *Phytophthora* infection. The infection caused by *P. palmivora* isolate, KL/CO-8 was the highest on CGD, LO and GBGD and lesion sizes did not vary between the three accessions. Similarly COD, D x T (COD x WCT), Benaulim and T x D were also equally susceptible to KL/CO-8. The highest infection caused by *P. palmivora* isolate, KL/CO-1 was observed on T x D and LO. LO was also found to be highly susceptible to the infection by *P. palmivora* isolate, KL/CO-78, *P. meadii* and also to *P. capsici.* COD and MYD were also equally susceptible to KL/CO-78 and the rate of infection did not vary from that on LO. *P. palmivora,* KL/CO-78 did not take up infection on Benaulim. MGD was very less susceptible to all three isolates of *P. palmivora.*

The rate of infection caused by *P. meadii* in COD, T x D and LO did not vary and these accessions were more susceptible to *P. meadii* than other seven accessions. The rate of infection caused by *P. meadii* was very low on all other accessions and CGD did not take up infection even seven days after inoculation of the detached leaf lets. WCT and T x D were highly and equally susceptible to *P. nicotianae* infection compared to other accessions. The rate of infection caused by this species was the highest on WCT and T x D followed by LO and GBGD. The rate of infection caused by *P. nicotianae* was the lowest in MGD and Benaulim. Among the 10 accessions screened, LO, MYD and T x D were only susceptible to *P. capsici* infection. Other accessions did not take up infection even though wound inoculated and found to be the least pathogenic. This technique developed for inoculation was found to be very useful, simple and easy technique.

The mean disease index calculated from the size of the lesions caused by the six selected isolates of *Phytophthora* spp. on 10 coconut accessions revealed that, in general, LO and T x D were highly susceptible to *Phytophthora* infection. Among the dwarf cultivars, COD, GBGD and MYD were more susceptible. The lesion sizes caused by all the four *Phytophthora* species were the smallest in MGD and *P. capsici* did not cause any infection in this cultivar, indicating the high level of resistance of MGD to *Phytophthora* infection.

A preliminary study was conducted to assess the susceptibility of few cocoa accessions to *Phytophthora* species isolated from cocoa. The lesion sizes caused by the two isolates of *P. palmivora* (AP/CA-334, KL/CA-216) and one isolate each of *P. citrophthora, P. capsici, P. meadii*

and *P. tropicalis* when artificially inoculated on detached cocoa pods of seven different cocoa accessions are presented in Fig. 10. The study revealed the variability in virulence of the five species as well as the two isolates of *P. palmivora.* Lesion size on pods of different accessions varied with the isolate and accession. *P. palmivora* was found to be more virulent than other four species. Among the two isolates of *P. palmivora,* the canker isolate, AP/CA-334 was more pathogenic than the black pod isolate. *P. meadii* was the least pathogenic species. The lesion size caused by canker isolate of *P. palmivora* (AP/CA-334) was more than by KL/CA-216 on all the seven accessions. The accessions, ICS-6 x SCA-6, II-67 x NC-29/66, NC x 45/53 and II-67 were more susceptible to *P. citrophthora* than the *P. palmivora* isolate, KL/CA-216 (black pod isolate). *P. tropicalis* was more pathogenic on pods of II-67 x NC-29/66 than both *P. palmivora* isolates and *P. citrophthora.*

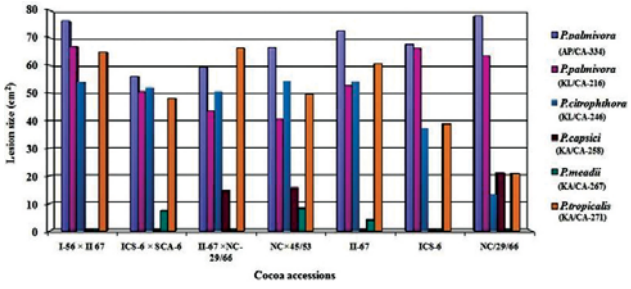

Fig. 10. Pathogenic variability of six selected isolates of *Phytophthora* species inoculated on pods of seven cocoa accessions

Accession II-67 was more susceptible to *P. tropicalis* than *P. citrophthora* and KL/CA-216 isolate of *P. palmivora. P. tropicalis* was also more pathogenic on pods of I-56 x II-67 and NC-29/66 accessions than *P. citrophthora.* In general, *P. tropicalis* was more pathogenic than *P. citrophthora. P. capsici* did not take up infection on pods of four accessions *viz., I*-56 x II-67, ICS-6 x SCA-6, II-67 and ICS-6. Similarly, the accessions I-56 x II-67, II-67 x NC-29/66, ICS-6 and NC-29/66 were resistant to *P. meadii* infection. Among the seven accessions tested, the accession NC x 45/53 was only susceptible to the infection of all six isolates. Thus, there was marked variability in virulence of the six isolates when inoculated on pods of seven accessions which was evident from the distinct differences in lesion size.

Field planting of promising cocoa grafts

The cocoa accession less susceptible to black pod disease in Thrissur district with negligible lesion size on pod surface under natural conditions during 2009 did not show any natural infection during 2010. This accession was multiplied by soft wood grafting and planted at CPCRI, RC, Kidu for further evaluation.

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a. *PHYTOPHTHORA* DIVERSITY

Collection and maintenance

Large number of *P. infestans* infected potato samples were collected from different geographic locations *viz*., Himachal Pradesh, Uttarakhand, Uttar Pradesh, Punjab, Bihar and Meghalaya. Over 100 isolates were purified on rye agar medium and maintained. Isolated DNA from 100 isolates.

Genotype differentiation and fingerprinting of *P. infestans* using SSR markers

Over 90 isolates collected from Himachal Pradesh, Uttarakhand, Uttar Pradesh, Karnataka and Tamil Nadu were genotyped using SSR primers. Out of 12 published SSR markers only two markers (Pi04 and Pi63) showed better polymorphism in the Indian population of *P. infestans*. Three alleles were observed with primer Pio4 (Fig. 11). Among the three alleles, 174 bp size locus was observed in most of the isolates.

Mt haplotype analysis for lineage determination

A subset of population comprising 70 isolates collected from Himachal Pradesh, Meghalaya, Assam, Tamil Nadu, Karnataka, Uttar Pradesh and Uttarakhand were analysed with primer F2-R2 (digested with *Msp*I) and F4-R4 (cut with *Eco*R1) for mitochondrial haplotyping which revealed that all the isolates belonged to the Ia haplotype (Fig. 12). Another set of population consisting of potato (20) and tomato (10) isolates were also analyzed with same primer sets and results revealed that both the isolates belonged to Ia haplotype as well. It tends to suggest that the new population which was introduced during 2002 is on the rise and has displaced the old population (Ib) in most of the regions.

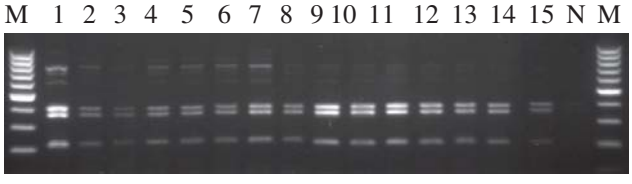

Fig. 12. Mt haplotyping with primer F4F4 and F4R4 digested with *Eco*R1, Lane: 1-15 *P. infestans* isolates, M: 100bp, N: Negative control

b. DIAGNOSTICS

Detection and quantification of *P. infestans* in host tissues

Real time PCR quantification was carried out to determine the *P. infestans* load in the host tissues. Sampling was performed at different distance from

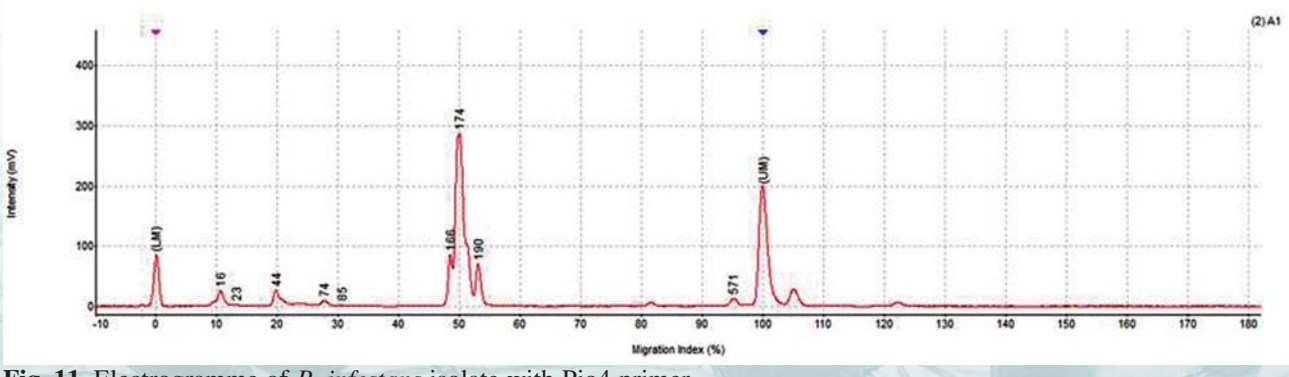

Fig. 11. Electrogramme of *P. infestans* isolate with Pio4 primer

the point of inoculation (5, 10, 15, 20 and 25 mm away from infected area) and at different time intervals (24, 48, 72, 96 and 120 h of post inoculation). A maximum of 2.98 ng of *P. infestans* load was estimated at 5 mm away from the symptomatic area while minimum 0.03 ng load was estimated 25 mm away from symptomatic area. This indicates that the *P. infestans* mycelium can spread the host tissue 25 mm away from the symptomatic area. Similarly the quantification was estimated at different time intervals. Minimum pathogen load (0.14 ng) was estimated at 24 h of post-inoculation while highest load (2.29 ng) was estimated at 120 h of post-inoculation.

Development of SCAR marker for detection of *P. infestans*

Twenty OPA series RAPD (random amplified polymorphic DNA) primers were screened to select a specific primer for the development of a SCAR marker for detection of *P. infestans*. PCR reactions were carried out with *P. infestans* (A1 and A2 strain), other *Phytophthora* species (*P. cactorum*, *P. colocasiae*, *P. capsici* and *P. palmivora*) and other fungal pathogens of potato (*Fusarium* sp., *R. solani*). Out of twenty OPA series primers, only OPA-12 primer gave the specific 1.5 kb amplicon in both the strains of *P. infestans*, while it was absent in other *Phytophthora* species as well as other potato fungal pathogens. Amplified products obtained with primer OPA-12 were sequenced and matched with NCBI database resulting in maximum similarity with *P. infestans*. From this sequence ten primers were developed (Primer-blast 3plus software), out of which only one primer PITH2-F/PITH2-R produced the desired amplicon in both the strains of *P. infestans* which was absent in other *Phytophthora* species and fungal pathogen of potato (Fig. 13). This PCR based assay enabled detection of *P. infestans* from host tissues.

c. EPIDEMIOLOGY

Development of decision support system

Need based application of fungicide was tested with the help of Decision Support System developed for the purpose.

Effect of elevated temperature on host resistance, fungicides efficacy and aggressiveness of *P. infestans*

An *in vitro* study on the efficacy of fungicides at different temperatures (10^{0}C and 15°C) was conducted using fungicides like dimethomorph

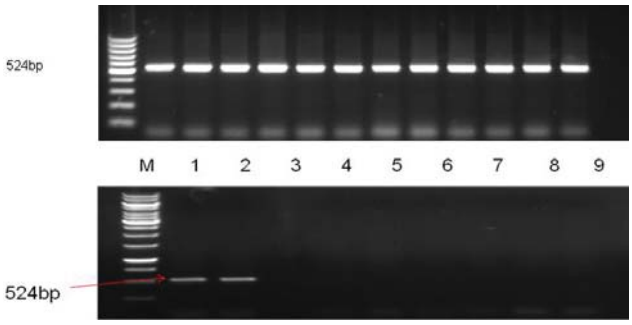

Fig. 13. PCR amplification with PITH2-F/PITH2-R primer. *Top:* Shimla isolates of *P. infestans, Bottom: Phytophthora* spp. and other fungal spp. Lane 1 and 2 - *P. infestans*, 3 - *P. palmivora*, 4 - *P. cactorum*, 5 - *P. colocasiae*, 6 - *P. capsici*, 7 - *F. solani*, 8 - *R. solani,* M: 1 Kb

(0.2%), cymoxanil (0.3%- cymoxanil + mancozeb), mancozeb (0.2%) and Ridomil (0.25% -metalaxyl + mancozeb). Potato leaves were detached at regular interval and challenge inoculated with *P. infestans*. After 14 days of spraying, no degradation was recorded in any fungicides compared to control (7.02 & 9.80 cm^2) at both temperatures.

Aggressiveness of *P. infestans* was tested at different temperature regimes (15^{0}C, 18^{0}C and 20^{0}C) and the highest lesion area (15.07cm^2) with lowest incubation period (< 30 h) was observed at 20^{0}C.

d. GENOMICS

Silencing of *RXLR* effector gene (*Avr3a*) of *P. infestans* in transgenic background for resistance development

Bioassay of the transgenic lines was done in a glasshouse by challenge inoculating (35 days old plants) with *P. infestans* (3.5 x 10^4 sporangia/ml). Based on disease scoring, five lines from siRNA transgenic lines (K. Pukhraj SI2AS1 2086, K. Pukhraj SI2AS1 2173, K. Pukhraj SI2AS1 2155 and K. Khyati SI2AS1 1037) and three lines from amiRNA transgenic lines (amiR2.1153, K.Khyati amiR4.4091 and amiR4.5002) were selected. The disease severity ranged from 15-20% in transgenic lines as compared to 75-85% in non-transgenic lines. Real time PCR analysis of *Avr3a* gene in transformed potato line (K. Pukhraj SI2AS1 1037) and non – transformed control plants confirmed the silencing of the *Avr3a* gene upon *P. infestans* challenge inoculation (Fig. 14).

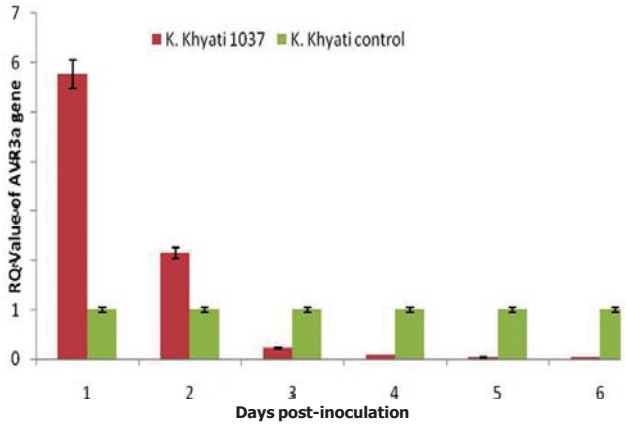

Fig. 14. Relative expression of Avr3a gene in transgenic K. Khyati SI2AS1 1037

Whole genome sequencing of *Phytophthora infestans* A2 mating type

The whole genome sequencing of A2 mating type, which is a new aggressive population to India, was done using GS FLX 454 pyrosequencing platform. Sequence analysis for annotation, SNP detection, comparison with already published sequence and two other *Phytophthora* spp. genomes is under progress.

e. HOST RESISTANCE

Allele mining for late blight resistance gene from *Solanum* species

Allele mining was done to investigate the late blight resistance *R* genes in the wild/semi-cultivated *Solanum* species by sequencing-based allele mining approach using known primer pairs (53 nos.) from the literature. A total of 44 *Solanum* species were screened for the late blight resistance in the *in vitro* chamber under controlled environmental conditions by challenge inoculation of *P. infestans*. Eighteen species were found highly resistant to late blight. PCR amplification was carried out using 53 primer pairs corresponding to different late blight resistance R genes following optimized protocols including annealing temperatures (Ta). As a result, all the species except three species showed amplification for presence of *R* genes by different primer combinations. In addition, 20 cultivated *Solanum* species comprising a mapping population of late blight resistance were screened for the presence of *R* genes using the 53 primer pairs which showed amplification of all the primers. Thus, in total 38 (18+20) *Solanum* species showed presence of 53 different *R* genes, which will be targeted for *R* genes allele mining through sequencing.

Validation of molecular markers for R-genes (validation of PCR markers in late blight differentials and screening of parental lines/ varieties)

Hybridization was attempted to combine *R1* and *R3a* genes in single host background. Seeds were extracted from six successful crosses *viz*., K. Jyoti × CP-4055, SM/92-338 × K. Girdhari, K. Chipsona-3 × CP 4043, HR 5-2 × K. Himsona and CP 4045 × CP 1945 which were raised under controlled glass house conditions. Genomic DNA was isolated from a segregating population of about 280 genotypes for identifying lines with stacked genes through MAS. About 15 clones were identified possessing both R1 and R3a genes through MAS. Besides, 20 genotypes with PVY resistance (*Ryadg* gene), 10 genotypes with PCN resistance (*HC*, *H1*genes) were also identified. Detached leaf bioassay was done for late blight resistance in selected clones along with parents and controls.

QTL mapping underlying resistance to late blight in a diploid potato population of *Solanum spegazzinii* × *S. chacoense*

Mapping of QTL for resistance to late blight in a diploid mapping population of 126 F_1 of *Solanum spegazzinii* (susceptible) × *S. chacoense* (resistant) was done. Area under disease progress curve (AUDPC) value of the mapping population displayed quantitative variation for late blight resistance by *in vitro* whole plant and detached leaf assays. Out of 64 AFLP primer-pairs combination and SSR marker, a total of 209 significant AFLP loci were placed onto 12 linkage group of potato covering total map length of 6548.1 cM. In QTL analysis for resistance to late blight for the AUDPC dataset of *in vitro* whole plant assay, interval-mapping analysis identified two QTL (LOD> 2.5) located on linkage groups IX and X (Fig. 15), which explained 14.70 and 3.40% variation, respectively. The present study revealed the presence of potential new genetic loci in this diploid potato family contributing to quantitative resistance against late blight.

f. DISEASE MANAGEMENT

Characterization of biosurfactant compounds

Analysis of crude biosurfactant was carried out by high performance liquid chromatography and mass spectroscopy from CDRI (Central Drug Research Institute), Lucknow. Four compounds were identified as rhamnolipid congeners based on their molecular formula and molecular weights. The

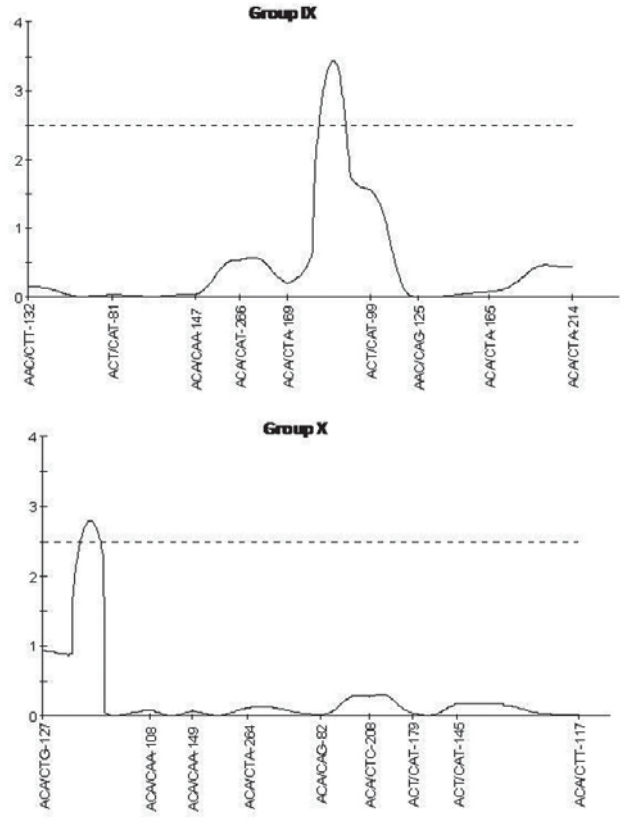

Fig. 15. Late blight resistance QTL mapping on linkage group IX and X in the 126 progenies of cross between *Solanum spegazzinii* × *S. chacoense*

mass spectra of these four rhamnolipid congeners are Rha-C_{12}-C_{14} peak value (m/z 584), Rha-Rha-C_{10}-C_{10} peak value (m/z 650), Rha-C_{12}-C-$_{12\text{-}CH3}$ peak value (m/z 574) and Rha-$C_{12.1}$-$C_{10.CH3}$ peak value (m/z 544). Other compounds with their peak values (682.8, 681.9, 708.8 and 935.2) could not be identified since any matching real value were not available in literature. In all four rhamnolipids congeners were identified. These were present in abundance in the crude biosurfactant obtained from the *Pseudomonas aeruginosa*. As confirmed by LC-MS analysis, the rhamnolipid produced by *P. aeruginosa* is a mixture of different rhamnolipidic congeners (mono-rhamno-mono-lipidic, mono-rhamno-di-lipidic and di-rhamno-di-lipidic). The mono-rhamno-di-lipidic congener had the highest relative abundance of M^- and $[M–H]^-$.

Isolation of novel microorganisms with activity against *P. infestans*

Eleven new bacteria were isolated from soil and screened for biosurfactant activity by using different biosurfactant assays. Results revealed that none of the isolate collected from agricultural soil had good biosurfactant potential.

Extraction and mass collection of rhamnolipid for formulation development

Rhamnolipid secreted by *P. aeruginosa* was collected (Kim *et al*. 1997) for formulation development. The rhamnolipid based formulation was developed in different solvents. Stock formulation of 5% rhamnolipid was prepared and used in all experiments. The detached leaves experiment was conducted to evaluate the phytotoxicity and biocontrol effect of rhamnolipid based formulation on different concentration (0.0025 to 0.5%). Results revealed that at 0.4% concentration and above showed phytotoxicity. However, below 0.15% it was not able to control the disease. The highest control was observed at 0.2- 0.3% up to five days of inoculation on detached leaves against control (4.90 cm^2).

CENTRAL TUBER CROPS RESEARCH INSTITUTE, THIRUVANANTHAPURAM

Principal Investigator:

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Co-investigators:

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a. *PHYTOPHTHORA* DIVERSITY

Genetic diversity of *P. colocasiae* using AFLP markers

Regionwise variability in *P. colocasiae* population was analysed by AFLP markers to understand the genetic diversity in *P. colocasiae* populations from different parts of India. *P. colocasiae* isolates were grown in potato dextrose broth medium (PDB) and their DNA was isolated. AFLP analysis was performed as described by Vos *et al*. (1996) with suitable modifications. After pre-screening of 36 primer pairs, seven selective primer pairs were chosen for this study (Table 4) and amplifications were performed in Biorad C1000 thermal cycler AFLP analysis produced a large number of reproducible and unambiguous markers for fingerprinting the isolates of *P. colocasiae*.

All clearly detectable, polymorphic AFLP bands were scored for their presence (1) or absence (0) by visual observation. A dendrogram was constructed using genetic similarity matrices to display relationships between isolates using the Nei and Li distance (1979) according to the unweighted pair group mean algorithm using the TREECON software package version 1.3 (Van de Peer 1997). The relative support for the different groups and stability of the dendrogram was assessed by bootstrap analysis (2000 replicates).

Allelic frequencies of AFLP marker were used separately to estimate the percentage of polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles (AE), observed heterozygosity (HO), and expected mean heterozygosity (HE) with respect to Hardy–Weinberg equilibrium (Hedrick 2000) using the computer program POPGENE 32 (Yeh and Yang 1999). Population genetic parameters of *P. colocasiae* isolates based on AFLP data is summarized in table 5. The observed number of alleles (N_A), effective number of alleles (N_E) and Nei's gene diversity values varied among

Table 4. Attributes of AFLP primers used in this study

Primer	Sequence (5'-3')	No. of bands scored	No. of polymorphic bands	Mean no. of bands	Polymorphism (%)
E+AG/T+AA	CTC GTA GAC TGC GTA CC AG/ TACTCAGGACTGGCAA	95	95	30.9	100
E+AT/T+AC	CTC GTA GAC TGC GTA CC AT/TACTCAGGACTGGC AC	52	50	18.0	96.1
E+AG/T+AT	CTC GTA GAC TGC GTA CC AG/TACTCAGGACTGGC AT	60	59	16.5	98.3
E+AC/T+AT	CTC GTA GAC TGC GTA CC AC/TACTCAGGACTGGC AT	40	40	7.2	100
E+GC/T+TC	CTC GTA GAC TGC GTA CC GC/TACTCAGGACTGGC TC	77	77	22.0	100
E+GA/T+GT	CTC GTA GAC TGC GTA CC GA/TACTCAGGACTGGC GT	51	51	14.2	100
E+AC/T+AC	CTC GTA GAC TGC GTA CC AC/TACTCAGGACTGGC AC	56	56	17.7	100
	Total	431	428	126.5	-
	Average	61.5	61.1	18.1	99.2

population studied. The population distance tree based on Nei's gene diversity indices is presented in Fig. 16.

The evidence for recombination was evaluated by performing linkage disequilibrium tests. The

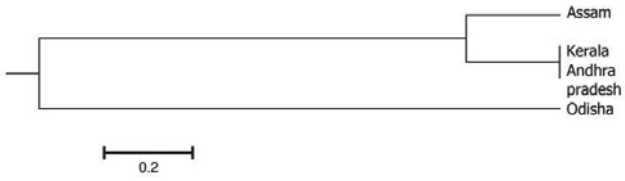

Fig. 16. Population genetic tree for four population of *P. colocasiae* based on Nei's distance

standardized index of association (*rBarD*) statistic (Agapow and Burt 2001) was used to estimate linkage disequilibrium (LD) in each population (Agapow and Burt 2001). The null hypothesis (*rBarD* = 0) can be rejected when the observed *rBarD* < 0.001, and it can be assumed that the sampled isolates probably originated from a population with a clonal mode of reproduction (Agapow and Burt 2001).

The similarity matrix was also used to perform a hierarchical analysis of molecular variance (AMOVA) (Excoffier 1992) by using FAMD Software version 1.25 (Schluter and Harris 2006). AMOVA based on AFLP data shows that a high percentage of the total genetic diversity of *P. colocasiae* populations in this study were distributed on a small spatial scale with 85% of the genetic diversity distributed within populations and only 14% among population (Table 6). The coefficient of genetic differentiation among populations (G_{ST}) was 0.091 which supports the AMOVA analysis indicating only limited genetic diversity among populations and significant diversity within populations. The pairwise Ö statistics were 0.147 indicating populations are considerably differentiated. The estimate of gene flow (*Nm*) among populations was 4.97.

The observed *rBarD* for four regions *viz.,* Assam, Kerala, Andhra Pradesh and Odisha were 0.0386 (P < 0.050), 0.0290 (P< 0.100), 0.0203 (P< 0.050), 0.0164 (P< 0.033), respectively. The result indicates that the *P. colocasiae* population has a recombination mode of reproduction.

The genetic relationship among 25 isolates of *P. colocasiae* was analyzed by 7 AFLP primer combinations on the basis of Nei and Li distance (Nei and Li 1979). Based on an UPGMA clustering algorithm, the genotypes were grouped into two major clusters (Fig. 17) with high bootstrap values. Cluster I formed the major group in 22 isolates while cluster II had 3 isolates. Isolates were grouped irrespective of their geographical origin and displayed a high level of genetic diversity among them. The cophenetic correlation coefficient between dendrogram and the original similarity matrix was significant (*r* = 0.816) markers

Table 5. Population genetic parameters of *P. colocasiae* isolates

Population code	Polymorphic bands	PPB[a](%)	N_A [b]	N_E [c]	H [d]	I [e]
Assam	351	81.44	1.814±0.389	1.355±0.293	0.229±0.155	0.362±0.218
Kerala	391	90.72	1.907±0.290	1.403±0.266	0.261±0.138	0.412±0.188
Andhra Pradesh	372	86.31	1.863±0.344	1.381±0.278	0.247±0.145	0.390±0.201
Odisha	285	66.13	1.661±0.473	1.315±0.301	0.202±0.167	0.316±0.245
Total	350	81.15	1.993±0.083	1.385±0.219	0.261±0.110	0.420±0.140

[a] Percentage of polymorphic bands (PPB), [b] Observed number of alleles (*NA*), [c] Effective number of alleles (*NE*), [d] Nei's gene diversity (*H*), [e] Shannon's information index (*I*)

Table 6. Analysis of molecular variance (AMOVA) for 25 isolates of *P. colocasiae*

Source	df	SSD	φ statistics	Variance components	Proportion of variation components (%)
Among population	2	1.33	0.147	0.049	14.71
Within population	22	6.31	-	0.287	85.28
Total	24	7.65	-	0.336	-

df - degrees of freedom; SSD - sums of squared deviations

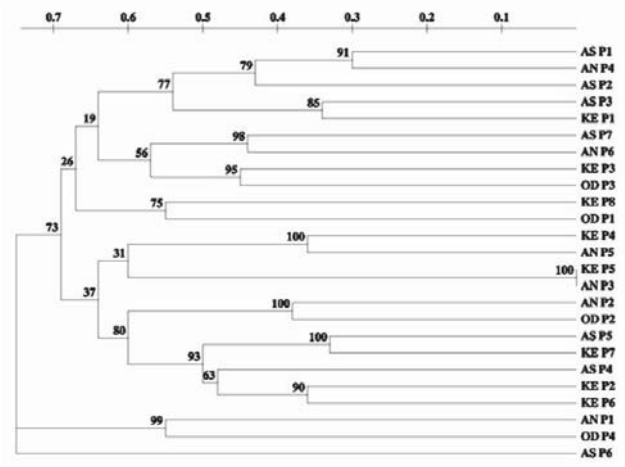

Fig. 17. UPGMA dendrogram depicting genetic relationships in 25 isolates of *P. colocasiae* based on AFLP data. Numbers at node represents bootstrap values (2000 replicates). (AS-Assam, KE- Kerala, AN- Andhra Pradesh, OD- Odisha)

supporting a good degree of confidence in the association obtained for 25 isolates of *P. colocasiae*.

The genetic similarity estimates obtained through AFLP analysis displayed profound genetic variation among isolates. Isolates from different fields shared a common clade which supports the fact that migration events are quite common in populations of *P. colocasiae*. The presence of larger than expected AFLP variation in isolates of *P. colocasiae* suggests that genetic recombination (or less likely hybridization) is at least possible in this oomycetes. A high level of recombination is suggested by the low index of association and we speculate that mitotic recombination events greatly contribute to the variation in *P. colocasiae* population.

Evidence of genetic diversity in *P. colocasiae* isolates obtained from fine spatial scale

A study was undertaken to analyze the diversity of *P.colocasiae* isolates obtained from fine spatial scale through cultural and molecular methods. Isolates of *P. colocasiae* used in this study were obtained from multiple leaf blight lesions present on a single taro leaf. Two-three months old taro leaves showing multiple lesions were collected on a random basis for the same field (approx. 800 m^2) of the Institute. *P. colocasiae* isolates from each lesion was treated individually, and grouped into populations according to their source leaves. A total of 41 isolates comprising four population were obtained and named as pop A (*n*=9), pop B (*n*=11), pop C (*n*=11) and pop D (*n*=10). Each isolate was stored at 20°C in 50% glycerol (long-term storage) and at 15°C on carrot agar (CA) slants in the dark (short-term storage).

Colony morphology: Isolates of *P. colocasiae* were analyzed for any morphological variation on PDA and CA plates. All the isolates tested had a similar growth pattern within individual media used. They depicted the white cottony appearance with concentric rings on the PDA and sparse uniform growth on CA plates. There was no distinguishable morphological pattern for any isolate. This suggests that all the isolates shared a common genetic base with respect to colony morphology.

Pathogenicity assay: Modified floating disc method was used to check the pathogenicity. All isolates were found to be virulent and reproduced typical leaf blight symptoms. The isolates initiated lesion development after three days of inoculation and the inoculated sites showed water soaked lesions at the beginning which turned brown upon the progression of the disease. There was no variation in lesion development among the isolates upon visual inspection. This suggests that all the isolates were similarly aggressive and shared common pathogenicity characters.

Determination of mating type: The mating type was determined by pairing each unknown isolate with the isolate of a known A1 (98-111) and A2 (98-35a) mating type on CA medium at 3 cm apart. After incubation at 28ºC in darkness for 4 weeks, agar blocks were examined microscopically. An isolate was designated to be mating type A1 if oospores were present when paired with a known A2 tester and vice-versa. The solo culture of each isolate was examined for oospore formation as a control. The positive control was a cross between two tester isolates of opposite mating types. The test was replicated two times. All isolates of *P. colocasiae* tested were of A1 mating type. Oospores were produced only when plates of each isolate included the tester isolate of the A2 mating type.

ITS characterization: Genomic DNA was isolated and the target DNA was amplified using the universal primers ITS1 (52 -TCCGTAGGTGAACCTGCGG-32) and ITS4 (52 -TCCTCCGCTTATTGATATGC-32) (White *et al.* 1990). The PCR products were extracted using a QIAquick Gel extraction kit and cloned into the pTZ57R/T vector and sequenced at Rajiv Gandhi Centre for Biotechnology (RGCB),

Thiruvananthapuram. The nucleotide sequences obtained were processed, aligned using ClustalW (Thompson *et al*. 1994) and a phylogenetic study was carried out (Tamura *et al*. 2011). Sequences were analyzed to determine the relatioships between isolates by the neighbor-joining method (Saitou and Nei 1987) using Maximum Composite Likelihood model. Bootstrap values were generated using 2000 replicates. Only those regions common to all the isolates were used for analysis (ITS1, 5.8S gene and ITS2). Number of haplotypes (H), haplotype diversity (HD), nucleotide diversity (π), and nucleotide substitution rate were calculated for each using DnaSP version 5.10.1 (Librado and Rozas, 2009).

Amplification with ITS primers (ITS1 - ITS4) yielded ~850 bp product in all isolates studied. The ITS sequence analysis revealed 97–99% nucleotide sequence homology with each other and 95–99% similarity among the isolates of *P. colocasiae* available in the GenBank database. Alignment of sequences using Clustal W revealed considerable variation in all the isolates examined. Variations ranged from single base pair changes to multiple changes representing deletions and insertions. More sequence variation was evident in the ITS1 region with only a few short regions showing complete homology across all isolates examined, whereas in ITS2 region the sequences were more conserved, with many regions of complete homology interspersed with variable regions. The nucleotide polymorphism defined twelve 'haplotypes' and the representative sequences are deposited in Genbank under accession numbers JX134643-JX134654. The number of haplotypes, haplotype diversity and nucleotide diversity was relatively high in all the sequences analyzed. The highest nucleotide diversity was seen in population B while the remaining population shared similar values for various parameters analyzed (Table 7). Phylogenetic tree clearly projected the variation among isolates of *P. colocasiae*. No isolate from the same leaf was grouped together indicating they are genetically diverse.

RAPD analysis: A set of 12 screened random decamer oligonucleotide primers were used for RAPD analysis of above populations. Distribution of amplification fragments was unique for each primer. To ensure credibility in scoring, all markers were scored at least twice. All the bands were distinct and reproducible. The 12 primer combinations amplified 198 reproducible fragments ranging in size from 200 to 1800 bp, of which 193 (97.9 %) were polymorphic (Table 8). When fingerprints of these isolates were compared, some bands common to the majority of isolates were observed, while others were unique to one or few isolates.

Genetic diversity varied among populations with the *PPB* values ranging from 54.04% (Pop D) to 90.91% (Pop A), with an average of 73.73%. The average Nei's gene diversity (*H*) was estimated to be 0.265 within populations and 0.329 for the pooled population. The observed number of alleles (N_A) and the effective number of alleles (N_E) and Nei's gene diversity varied among population. Among the four studied population, population D exhibited the lowest levels of diversity (*PPB* = 54.04%; *H* = 0.2065; *I* = 0.3052; N_A = 1.5404; N_E = 1.3555), whereas population A showed the highest variability (*PPB* = 90.91%; *H* = 0.3187; *I* = 0.4743; N_A = 1.9091; N_E = 1.5565). The remaining population of B and C exhibited diversity from low to high.

Analysis of molecular variance (AMOVA) showed that a high percentage of the total genetic diversity of the four *P. colocasiae* populations in this study were distributed on a small spatial scale with 63.54% of the genetic diversity distributed within populations and less yet significant 36.45% among

Table 7. Summary statistics of DNA polymorphism in ITS1 region for four population of *P. colocasiae* obtained from multiple leaf blight lesions on taro leaf

Population	Sequences(N)[a]	Length[b] (bp)	π[c]	Θsites[d]	H[e]	HD[f]
PopA	6	840	0.024	0.023	6	1.00±0.096
PopB	7	840	0.176	0.236	7	1.00±0.076
PopC	6	840	0.041	0.050	6	1.00±0.096
PopD	8	840	0.034	0.043	8	1.00±0.063

[a]Number represents sequences analyzed from each population, [b]Length corresponds to the regions used for analysis (ITS1, 5.8S and ITS 2), [c]Nucleotide diversity, [d]Watterson's Theta per site, [e]Number of haplotypes, [f]Haplotype diversity

Table 8. Attributes of the primers used for RAPD amplification and number of bands per primer

Primer	Sequence 5'-3'	No. of bands scored	No. of polymorphic bands	Mean no. of bands	Polymorphism (%)
OPT6	CAAGGGCAGA	15	15	4.3	100
OPT7	GGCAGGCTGT	20	20	6.5	100
OPT13	AGGACTGCCA	19	19	6.9	100
OPG10	AGGGCCGTCT	14	14	5.7	100
OPG 12	CAGCTCACGA	17	17	5.9	100
OPG 16	AGCGTCCTCC	16	16	4.3	100
OPG 17	ACGACCGACA	13	13	4.5	100
OPG 18	GGCTCATGTG	15	15	5.9	100
OPG19	GTCAGGGCAA	21	21	8.2	100
OPA2	TGCCGAGCTG	12	12	4.5	100
OPA10	GTGATCGCAG	16	16	6.9	100
OPA15	TTCCGAACCC	20	15	5.4	75
	Total	198	193	69	97.9
	Average	16.5	16.0	5.75	97.9

populations. The coefficient of genetic differentiation among population (G_{ST}) was 0.2007, which supports the AMOVA analysis indicating only limited genetic diversity among populations and high diversity within population. The estimate of gene flow (*Nm*) among populations was 1.991 migrants per generation, obtained from the G_{ST} value. The Nei's genetic distances (*D*) between *P. colocasiae* isolates from four leaves ranged from 0.0866 to 0.9171, with an average of 0.5018. Similarly, the genetic identity (I_N) between *P. colocasiae* isolates from leaf samples varied from 0.0699 to 0.9325. A UPGMA cluster analysis of 41 individuals indicated that the samples from the same population did not form a distinct group (Fig. 18).

P. colocasiae populations had comparatively low rBarD values (0.1411, 0.0876, 0.0539 and 0.0837, respectively) which suggest that recombination events are common in these population. The cophenetic correlation coefficient between the dendrogram and the original distance matrix of the RAPD profiles was significant, with a high correlation value (r = 0.88, P = 1).

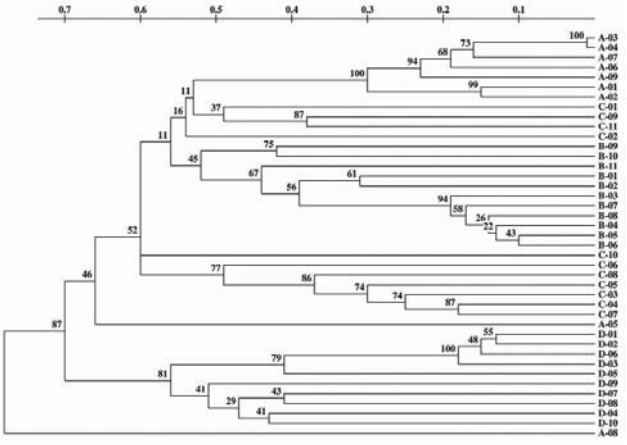

Fig. 18. Dendrogram (UPGMA) of 41 isolates of *P. colocasiae* isolates. The letter and digit represent the population code and isolate number from each population. The cophenetic correlation coefficient between the dendrogram and the original distance matrix of the RAPD profiles was significant, with a high correlation value (r = 0.88, P = 1)

b. DIAGNOSIS

Development and validation of *P. colocasiae* specific primers

DNA was extracted from pure cultures of *P. colocasiae* obtained from leaf blight infected samples (leaf and petioles), other *Phytophthora* species and bacteria according to previously standardized procedures. Genomic DNA of four selected *P. colocasiae* isolates (from different geographical origins) were amplified using the *Phytophthora* genus specific primers corresponding to the four target genes such as the *RAS*-like (Chen and Roxby 1996), *TRP1* (Karlowsky and Prell 1991), *GPA1* (Laxalt *et al.* 2002), *ASF*-like (Munakata *et al.* 2000) as previously described by Ioos *et al.* (2006). This

yielded a single approx. 210-bp product for (*RAS*-like, *TRP1*, *ASF*-like) and approx. 510 bp for *GPA1* in all the isolates. The amplified bands were eluted using Genelute Gel extraction kit, cloned using InsTAclone PCR cloning kit according to manufacturer's instructions and sequenced. The nucleotide sequences obtained were processed and analyzed using Geneious Pro software version 5.6. A homology search was performed with BLASTn module in NCBI. DNA sequence analysis of *P. colocasiae* isolates revealed considerable nucleotide sequence homology with similar gene sequence of other *Phytophthora* species available in the database.

The corresponding gene sequences available in the database were downloaded after BLAST search and aligned with the obtained *P. colocasiae* sequences using the Clustal W program in BioEdit software. Regions of dissimilarity between the consensus of *P. colocasiae* and other *Phytophthora* and fungal species were identified and potential primers specific for *P. colocasiae* were developed using the Primer Premier 6.0. To test the specificity of the primers, purified DNA from bacterial species (*Erwinia carotovora*), other *Phytophthora* species (*P. capsici*, *P. citrophthora*, *P. parasitica*, *P. araceae*, *P. meadii*, *P. nicotianae*, *P. infestans*, *P. cryptogea*, and *P. palmivora*), and isolates from other fungal species (*Fusarium solani*, *Colletotrichum capsici*, *Colletotrichum gleosporoides*, *Botryodiplodia theobromae* and *Rhizoctonia solani*) were amplified using the designed primer pairs. No amplifications were recorded with the tested bacterial, fungal, or *Phytophthora* species other than *P. colocasiae*, which indicated that no corresponding sites of the designed primer existed in the genomic DNA of the other tested organism. In addition, a primer blast was performed including all the organisms in the NCBI database to ascertain the specificity of the designed primers. No hit was recorded in all the trails using the four primer pairs targeting four genes *viz.*, *Ypt*1, *TRP*1, *ASF*-like and *GPA*1.

c. EPIDEMIOLOGY

Real time quantification of pathogen in infected propagules

Total genomic DNA was extracted from leaf blight infected samples collected from taro accessions. Real-time PCR reactions were run in triplicates on an Eppendorf realplex system. After a 10 min denaturation step at 95°C, samples were run for 35 cycles of 30 s at 95°C, 45s at 54.5°C and 1 min at 72°C. Serial dilutions of pure genomic DNA from *P. colocasiae* were used to trace a calibration curve, which was used to quantify plant and fungal DNA in each sample. Usual real-time quantitative PCR controls were performed to check for the linearity of amplification over the dynamic range. Standards showed that the primer pairs used allow reliable DNA quantification over a very wide dynamic range (10.4 ng of initial DNA concentration), providing an excellent sensitivity to this technique. Two different DNA extraction methods on comparison showed that they both led to quantitative recovery of fungal and plant DNA. The lower DNA quality yielded in some instances did not interfere with the PCR reaction.

A simple and rapid method for zoospore generation in *Phytophthora colocasiae* using taro (*Colocasia esculenta*) leaf *in vitro*

The use of taro leaf in production of large numbers of zoospores *P. colocasiae* was studied *in vitro*. For this, young leaves of taro (cv. Sree Kiran, susceptible to leaf blight) was collected, surface sterilized and blot dried. A mycelial plug (5 mm dia) of *P. colocasiae* excised from carrot agar (3-5days) plate was inoculated inversely over the midrib of the leaf. A layer of wet cotton was covered over the plug and the leaf was also covered with wet cotton. The control leaf was treated with water agar. The leaf was placed in a petri dish over a layer of water and observed for the development of lesions. The leaves upon infection generated watery brown lesions as early as 48 hrs post inoculation, a typical characteristic of the disease observed under field conditions. The leaves after the development of lesions were cut into pieces, transferred to a petri dish flooded with water, incubated at room temperature and the leaf fragments were observed daily under microscope. Zoosporangia were produced within five days of incubation. The zoospores produced were calculated based on its number from a leaf segment used. The mean number of zoospores released/ml varied from 5 x 10^5 to 4 x 10^6. The zoospores germinated were further tested for the ability to cause infection in taro leaf. The method was rapid and resulted in high yield of zoospore concentration without the involvement of any chemicals and low temperatures. This method has the advantage of re-using the leaf fragments for the continued generation of zoospores and suspension can be used as a source of inoculum for the subsequent infections in fresh leaf samples.

Survival of *P. colocasiae*

Survival study was carried out on soil and leaf fragments (as described above). The zoosporangia (1 x 10^3) of *P. colocasiae* were mixed with sterile soil mositured with sterile double distillled water. In another method, an Eppendorf tube carrying an infected taro leaf with sterile distilled water was used. All the samples were incubated at 25±2°C. After every two weeks, the samples were tested for the pathogen survival by using the plating method and taro leaf as bait. Colony and lesion formation were observed. For survival study in soil, samples were taken weekly by removing 1 g soil, shaken vigorously for 30 s and centrifuged. The supernatant (20 µl) was plated on PDA. The samples did not yield any Phytophthora on plating. Lesion formation was almost similar from both samples on initial two samplings but afterwards the infection formation from soil was delayed and reduced when compared to the infection from leaf sample. Detection of the pathogen using PCR conditions has been initiated.

Development of rapid and efficient method for pathogenicity assay for *P. colocasiae*

Young leaves of taro (cv. Sree Kiran) were collected, surface sterilized, blot dried and cut into fragments of equal length (5cm x 5cm) and were allowed to float on their abaxial surface in a pertiplate (20 cm dia) flooded with water. Zoospore suspension (20 µl) from the most virulent isolates of *P. colocasiae,* was placed over the center of leaf fragments and a drop of plain agar was overlaid to maintain high moisture (Fig. 19). The plate was incubated at 25°C and observed daily for lesion development. The leaves started developing lesions 48-72 h of incubation depending upon the aggressiveness of the isolate. Floating the leaves over the water allowed the leaf to be in the fresh state during the incubation period. During infection, mycelial growth was observed over the lesions as in field conditions and results obtained showed a greater degree of correlation to that of the field disease scores. This clearly shows the reliability of the method and could be adapted for rapid and efficient screening of taro accessions.

e. HOST RESISTANCE

Screening for host resistance

Twenty five taro accessions maintained by CTCRI, Thiruvananthapuram, selected based on a preliminary evaluation of disease incidence levels

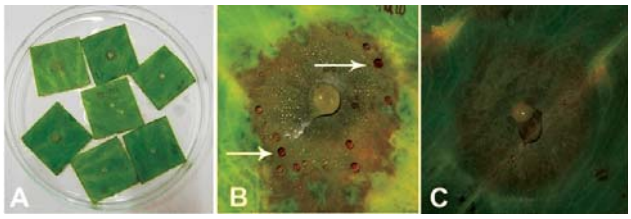

Fig. 19. Modified floating disc method for rapid pathogenicity assay in taro (*Colocasia esculenta*). (a) the experimental setup, (b) taro leaf disc showing typical symptoms of *P. colocasiae* infection. Note the formation of brown water soaked lesions (indicated by arrows) on the infected area, (c) taro leaf disc showing growth of *P. colocasiae* mycelia on the lesion developed area

in the field, were inoculated with the most virulent *P. colocasiae* isolate (PC 13-N) using the floating leaf disc method described previously. After two days of incubation at 25°C in the dark, the leaf discs were daily examined visually for disease symptom. Subsequently, the lesion diameter was calculated. The results categorized the accessions to varying levels of resistance. The leaves produced lesions ranging from 0.8 cm – 5.0 cm, similar to field conditions. The method was able to discriminate the levels of resistance present among the accessions and was highly reliable.

f. DISEASE MANAGEMENT

A pot trial has been carried out for testing the efficacy of bio-control agents (*Trichoderma harizianum* and *Pseudomonas* sp.) and a fungicide (Samarth) against leaf blight disease caused by *P. colocasiae*. There were 12 different treatments. Each pot was filled with around 20 kg of soil and treated with *Trichoderma* (@ 9 x 10^6 spores per gram soil) or *Pseudomonas* (@ 10^6 bacterial cells per gram soil) or fungicide (0.1%) as per the treatment. The plants were challenge inoculated with the zoospores of *P. colocasiae* after five months of planting and the disease incidence was recorded. Of the treatments, the least disease incidence was observed in soil treatment with *T. harzianum* but in contrast high yield was observed in control than other treatments. Even though a different range of incidence of disease occurred it was not having any influence in yield of the tubers which might be due to several conditions like the occurrence of disease after the maturation of tubers or through various environmental factors. Further studies are essential to conclude the role of bio-control agents/ fungicides against taro leaf blight disease.

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a. *PHYTOPHTHORA* DIVERSITY

Survey of apple orchards and incidence of collar rot disease

Periodical survey of apple orchards located in Chamba, Sirmour and Kinnaur districts indicated that incidence of collar rot disease (*Phytophthora cactorum*) varied between 2.6 - 18.8, 1.2 - 24.6 and 0.4 - 8.3 per cent, respectively. Maximum incidence was observed in Jattota (Shalooni block) and Baragarh (Tisha block, Chamba district), Leonana (Sirmour) and Lippa (Kinnaur district) of Himachal Pradesh. In total 156 soil/bark samples were collected from the different apple orchards.

Isolation of *Phytophthora* isolates

Fourteen isolates of *P. cactorum* and six isolates of *Pythium* were isolated from the soil/diseased bark samples collected from Chamba, Sirmour and Kinnaur apple growing areas. Studies on their morphology revealed that maximum size of sporangium and mycelium width was recorded in the isolate PC9 with whitish floppy radiate colony followed by 2, 7, 4, 6 and 13 respectively (Fig. 20). Isolate 3 and 5 produced creamish white colonies with slightly smaller sporangia. The size of oospore in all the isolates varied between 22-25 µm.

Virulence of the above isolates were tested on susceptible rootstock MM106 by excised twig

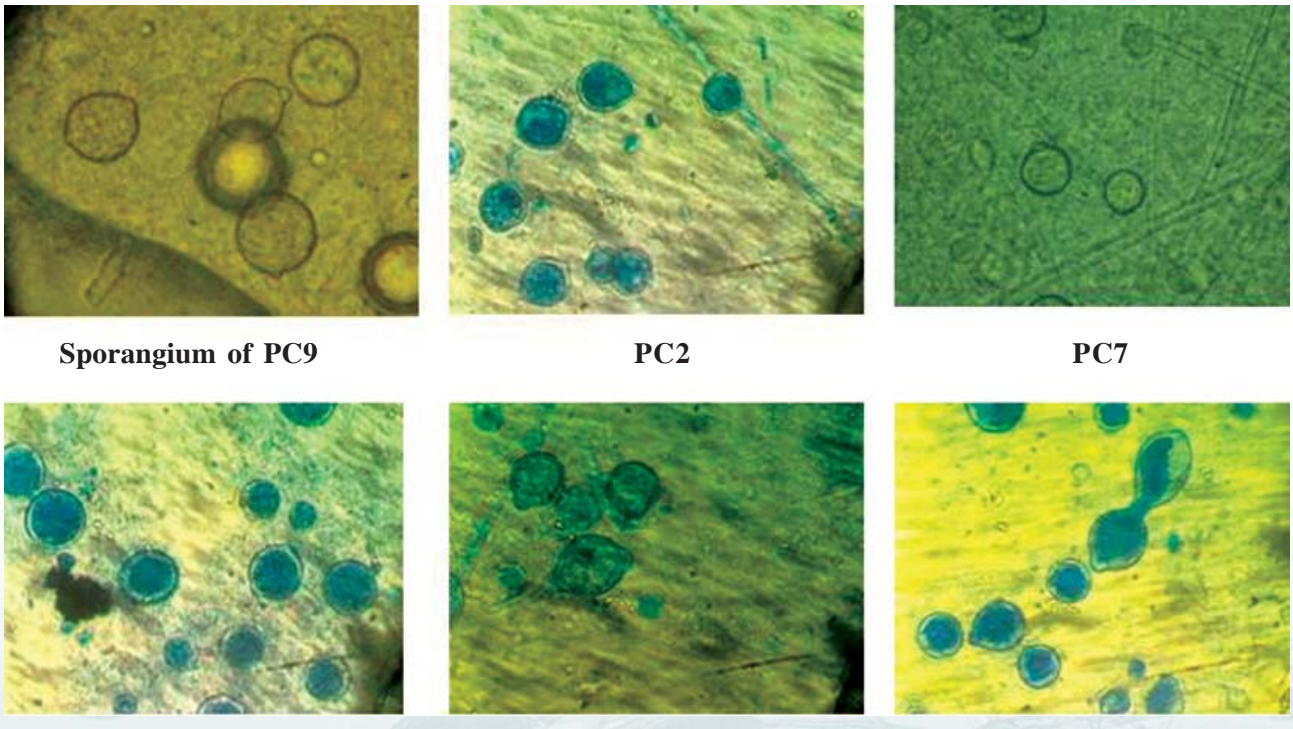

Fig. 20. Isolates of *Phytophthora cactorum* collected from Chamba, Sirmour and Kinnaur districts of Himachal Pradesh

method and data indicated that all the isolates produced different sized lesions. Isolate PC9 exhibited maximum lesion size (64.8 mm) followed by isolate 2, 7, 4, 13 and 6. On the basis of lesion size (>50 mm) six were designated as highly virulent, four as virulent, two as moderately virulent and two as less virulent. In addition, virulent strain produced dark brown lesions whereas less virulent produced light brown lesions. It was further observed that the isolates having larger sized mycelium and sporangia were more virulent and produced bigger lesion size.

b. EPIDEMIOLOGY

Disease progression studies

The incidence and progress of disease was recorded periodically under nursery condition (sick plot). A perusal of the data indicated that the disease first appeared in the first week (4 April) of April 2012, with mean minimum and maximum soil temperature ranging between 11.1 - 12.2°C and 23.5 - 26.1°C accompanied with soil moisture of 50.1 - 63.5 per cent (upto 5 cm) depth and frequent rains during two weeks preceding the appearance of disease. Similarly, mean minimum and maximum soil temperature of upto 10 cm depth varied between 12.6 -13.8°C and 21.1-23.2°C with soil moisture varying between 59.9 - 60.1 per cent during two weeks prior to the appearance of the disease. Further, the results indicated that disease increased with greater speed with the occurrence of minimum soil temperature ranging between 21.1 - 25.9°C and maximum temperature ranging between and 20.4 - 27.2°C up to 10 cm depth during April to July and reached its maximum in the second week of August 2012. The disease enhanced with faster speed with the prevalence of soil moisture ranging between 65.7-73.6 per cent.

In orchards, the disease was first noticed on 21 March 2012 with the occurrence of mean minimum and maximum soil temperature (at 5 cm depth) ranging between 6.1 - 9.1°C and 17.4 - 20.6°C accompanied with soil moisture of 50.8 - 68.4 per cent and frequent rains during two weeks prior to the disease appearance. Similarly, at 10 cm depth, the mean minimum and maximum soil temperature varied between 6.4 - 9.9°C and 18.3-21°C with the soil moisture ranging between 60.1 - 69.3 per cent in the two week prior to the onset of disease. The disease increased with greater speed when the minimum temperature and maximum soil temperature ranged between 21.1 - 25.9°C and 20.4 - 26.6°C, respectively, during the month of April-May and July-August and reached its peak in the second week of August 2012. The disease progressed faster when the soil moisture was between 68.2-80.2 per cent. Correlation studies indicated that disease is positively correlated with rainfall, soil moisture and minimum soil temperature and was negatively correlated with maximum soil temperature (Table 9).

Table 9. Correlation of different environmental factors with collar disease severity in apple

Factor	Correlation under conditions	
	Nursery	Orchard
Rain fall x PDI	0.34	0.13
Min. soil temp. 5 cm x PDI	0.81 **	0.22
Max. soil temp. 5 cm x PDI	-0.37	-0.09
Soil moisture 5 cm x PDI	0.35	0.27
Min. soil temp. 10 cm x PDI	0.69 **	0.05
Max soil temp. 10 cm x PDI	-0.33	-0.09
Soil moisture 10 cm x PDI	0.26	0.23
Min. soil temp. 20 cm x PDI	0.78 **	0.32
Max. soil temp. 20 cm x PDI	0.68 **	0.22
Soil moisture 20 cm x PDI	0.61	0.39 *

*Significant at 5% level, ** Significant at 1% level

Studies on rhizosphere microflora in sick and *Phytophthora* free soil

Fungal and bacterial microflora from the soil samples collected from healthy and diseased areas were estimated by using Rose Bengal supplemented PDA/ malt extract and nutrient agar medium, respectively. Results obtained indicated that *Phytophthora* containing soil sample had low population of *Trichoderma*, *Penicillium* and bacterial species whereas, *Aspergillus* and *Pythium* spp. were present in higher level and reverse was recoreded in non *Phytophthora* orchard soils. Studies undertaken during 2011 also indicated similar results.

c. HOST RESISTANCE

Screening of apple seedlings for resistance

The seeds of 25 pollinizer cultivars were collected, stratified and sown in pots containing sterilized soil in nursery. The inoculum of the target pathogen was added @ 2% (w/w) at four leaf stage in the pots and 60 g/m^2 under nursery conditions. The results obtained (Table 10) indicated that *Malus floribunda* showed maximum tolerance with minimum mortality of 12.8 and 10.2 per cent under

Table 10. Resistance level in different pollinizer cultivars of apple against collar rots under pot and nursery conditions

Cultivar	Seedling mortality (%)		Reaction
	Pot	Nursery	
Gold spur	64.9	68.8	HS
Red Gold	80.8	76.3	HS
Red Fuji	82.5	72.9	HS
Scarlet Gala	76.4	82.5	HS
Tydeman's Early Worcester	64.5	60.2	S
Mollies Delicious	56.3	59.1	S
Granny Smith	92.3	86.2	HS
Commercial	69.6	62.5	HS
Winter Delicious	20.6	17.1	MR
Golden Delicious	27.0	22.5	MS
Gale gala	78.3	71.8	HS
Royal Gala	62.9	53.5	S
Bray burn	14.4	10.8	R
Gloster	24.8	20.4	MS
Black Ben Davis	32.9	26.3	MS
McIntosh	90.3	86.4	HS
Spartan	72.4	70.8	HS
Summer Queen	52.7	56.2	S
Red June	48.4	42.3	MS
Lord Lambourne	38.9	48.1	MS
Golden Spur	72.1	70.7	HS
Jonathan	39.2	44.6	MS
Winter Banana	45.2	40.6	MS
Malus floribunda	12.8	10.2	MR
Star Crimson	17.8	14.3	MR
CD at 5 %	3.8	4.2	

pot and nursery conditions, respectively. It was followed by Bray Burn, Star Crimson, Winter Delicious, Gloster, Golden Delicious and Black Ben Davis Delicious. Other cultivars were less tolerant.

Adaptive trial on resistant rootstocks

A new trial was laid out with different applce rootstocks of M9, MM111 (resistant, M7 (moderately resistant) and MM106 (highly susceptible) at seven hot spot loctions *viz.*, Poojan, Sainj (Kullu), Ruhmani, Ruhanda (Mandi), Seema, Chirgoan and Dalgaon (Shimla). The trial laid out during last year at Poojan, Summa (Kullu), Gihiri, Chhatri (Mandi), Seema, Dalgaon (Shimla), indicated that resistant rootstocks performed well compared to susceptible rootstocks (MM106). In addition, these resistant rootstocks survived in sick soil under nursery condition at Kullu and Nauni.

Development of resistant rootstocks/ plants through somaclonal variations

Plant materials have been multiplied from the already identified *in vitro* shoot cultures of apple rootstocks (MM106 and MM111). Pure culture of *P. cactorum* was procured from Department of Mycology and Plant Pathology, UHF, Nauni, Solan for screening studies on somaclonal variants.

d. DISEASE MANAGEMENT

Isolation and evaluation of biocontrol agents

Fourteen fungal and 16 bacterial antagonists were isolated from the soil collected from two apple growing districts (Sirmour and Chamba) of Himchal Pradesh by using standard procedures (dilution plate, specific (TSM) medium and modified selective medium). *In vitro* evaluation of these antagonists indicated that *Trichoderma harzianum* - 2, 5, *T. viride* - 3, 4 and *T. virens* - 2 and *Penicillium funiculosum* were highly effective (percent growth inhibition 70.2-83.8). Similarly, *Bacillus* sp 2, 4, 7, *Pseudomonas* sp. 3, 8 and *Enterobacter aerogenes*- 1 were effective (PGI = 63.8, 74.6, 68.2, 71.3, 63.7, 72.7) against *P. cactorum*

The *in vitro* effective biocontrol agents (BCAs) were further evaluated under pot conditions. The stratified seeds were dipped in the biocontrol agents' suspension (cfu 5.2 x 10^4 /ml) for 15 minutes prior to sowing and seedlings were infected by addition of target pathogen at four leaf stage @2% (w/w). The BCAs were added separately as talc based formulation @2% (w/w) and 20 g wheat bran culture (cfu 5.2x10^4/g) at 14, 7 days before as well as on the date infection. Data on seedling mortality were recorded periodically up to 60 days from the first appearance of disease. The results indicated that addition of *T. harzianum*-5 provided and 82.6, 70.1 and 59.2 per cent control when applied 14, 7 days prior as well as simultaneous addition of BCAs and inoculum (0 day) respectively (Table 11). It was followed by *T. viride*-4, *T. virens*-2 and *Penicillium funiculosum*, in decreasing order.

Table 11. Evaluation of fungal biocontrol agents against collar rot under pot conditions

Fungal antagonist	Disease control (%)		
	14+	7+	0*
T. harzianum - 2	74.1	60.2	51.4
T. harzianum - 5	82.6	70.1	59.2
T. virens - 2	75.6	66.7	53.1
T. hematum - 1	68.2	59.8	48.1
T. viride - 1	73.5	60.1	50.8
T. viride - 4	81.6	68.5	54.1
Penicillium funiculosum	75.2	64.1	50.3
Control (untreated)	1.8*	0.5*	1.2*
CD 5 %	2.16	2.81	1.96

*both biocontrol agent and pathogen were added simultaneously
+addition of BCAs prior to challenge inoculation (days)

In another study, the stratified seeds were dipped for 15 minutes in suspensions (cfu10^8 /ml) of promising bacterial biocontrol agents and evaluated as described above. Further, these BCAs were individually added (cfu10^8 /ml @ 25ml/ pot) 15, 7 and 0 days prior to addition of pathogen @1% (w/w). The talc based formulation (1.6 x 10^{12} cfu/ ml) of each bacterial antagonist was also added @ 10 g/m^2 in the last week of April. The coconut coir based formulation @ 20 g/pot (cfu 1.2 x10^9/ml) of each BCA was also added. The collar rot pathogen was inoculated @ 60 g/m^2 in the first week of April to maintain the desirable inoculum threshold level. Data on seedling mortality was recorded periodically starting from first appearance of disease till 60 days (Table 12). The results indicated that addition of *Bacillus sp.-4* as highly effective in controlling collar rot disease under pot conditions and provided 79.2, 71.6, 67.6 PDC (percent disease control) at the addition of target pathogen (14, 7 and 0 days), respectively. It was followed by *Enterobacter aerogenes*-1, *Pseudomonas sp*-3 and *Bacillus sp*-7.

Studies on fungicide compatibility with BCAs

Two biocontrol agents (BCAs) *viz*., *T. viride-5* (TV5) and *Bacillus* sp.-11 (BS-11) were tested under pot conditions for their compatibility with metalaxyl MZ. BCAs were added individually (@ 2% bran/coconut coir culture + 0.2% talc based formulation) or in combination with metalaxyl MZ (0.25, 0.3%) at seven days prior to inoculation, simultaneously, and also seven and 12 days after inoculation with the target pathogen. The plants were infected by adding the pathogen grown on maize grain + potato slices medium @ 2.0 (w/w). Data on seedling mortality were recorded periodically till 75 days of first appearance of symptoms and percent disease control for each treatment was calculated. The study indicated that pre-inoculation treatments with either BCAs or metalaxyl MZ exhibited more disease control in comparison to their post inoculation applications (Table 13). Combined application of TV5 or BS-11 with metalaxyl MZ at 0.3 per cent provided

Table 12. Evaluation of bacterial antagonists against collar rots pathogen of apple under *in vitro* and pot conditions

Antagonist	Growth inhibition (%) of pathogen		Mean	Disease control (%) under pot conditions before (days) addition of pathogen inoculum		
	Dual culture	Culture filtrate		4	7	0
Bacillus sp - 2	62.1	65.5	63.8	60.1	57.4	50.8
Bacillus sp - 4	72.5	76.7	74.6	79.2	71.6	67.6
Bacillus sp - 7	67.3	69.1	68.2	68.2	63.1	60.9
Pseudomonas sp - 3	70.2	72.4	71.3	75.2	69.8	66.3
Pseudomonas sp - 8	62.2	65.2	63.7	66.5	52.7	49.2
Enterobacter aerogenes - 1	71.6	73.8	72.7	70.1	64.2	58.1
Control	80.0 *	79.5 *	79.7	1.3	0.6	0.8
CD at 5 %	1.03	1.89	1.46	2.96	3.05	2.67

Table 13. Effect of combined application of biocontrol agents and fungicides against apple collar rot (*P. cactorum*) disease under pot culture condition

Treatment	Concentration (%)	Disease control (%) after 45 days of inoculation (in treatments applied before/after pathogen inoculation)			
		7 days before	0 day**	7 days after	12 days after
Trichoderma viride-5 (TV 5)	2% (bran culture) + 0.2% (talc based culture)*	79.2	68.9	58.4	41.8
Bacillus sp.-11 (BS-11)	2% (coconut coir culture) 0.2% (talc based culture)#	80.1	72.1	60.7	50.2
Metalaxyl MZ	0.25	78.4	71.3	62.1	45.3
Metalaxyl MZ	0.3	91.5	80.9	70.2	54.8
TV5 + Metalaxyl MZ	A + 0.25	89.4	82.5	67.3	51.8
TV5 + Metalaxyl MZ	A + 0.3	95.6	85.6	72.6	56.0
BS11 + Metalaxyl MZ	B + 0.25	93.8	86.1	80.0	65.5
BS11 + Metalaxyl MZ	B + 0.3	97.6	91.6	84.8	69.8
TV5 + BS11 + Metalaxyl MZ	A + B + 0.25	98.9	91.1	86.4	68.1
TV5 BS11 + Metalaxyl MZ	A + B + 0.3	100.0	95.2	88.5	74.5
TV 5 + BS 11	A + B	89.6	81.5	75.6	62.1
Control	-	1.8	0.9	1.3	1.1
CD at 5%	-	2.75	2.96	2.67	3.19

* TV 5 cfu/g = 2.5 x 10^6 in talc based culture, TV 5 cfu/g = 2.0 x 10^3 in bran based culture
\# BS11 cfu/g = 9.1 x 10^9 in coconut coir based culture, BS11 cfu/g = 9.1 x 10^9 in talc based culture
** target pathogen and treatments were added simultaneously

higher disease control in comparison to separate applications.

Similar trial was conducted under field conditions in the nursery (sick plot) by adding the above BCAs, fungicides at 20, 10 before and 10 days after planting of apple seedlings and simultaneously at the time of planting. The bran and coconut coir cultures were added @150 g/m^2 along with 50 g talc based formulation of individual BCA. Fifteen apple seedlings were planted in each plot of 1 m^2 and each treatment was replicated thrice. Data on disease mortality was recorded periodically and final data on disease severity by following 0-5 disease rating scale was recorded in the month of December. Combined application of TV5, BS11 and metalaxyl MZ (0.3%) was highly effective to control collar rot infection (Table 14).

Similar studies were undertaken to find out compatibility of metalaxyl MZ with other effective biocontrol agents such as *T. harzianum*-15 (TH1 5) and *Bacillus sp.*-11 (BS-11) under nursery condition and results indicated that the combined effect of BCAs with metalaxyl provided 100.0, 92.6, 80.8 and 77.2 per cent disease control when applied 20, 10 before, 0 (simultaneously) and 10 days after inoculation. The pre-inoculation treatments proved more effective than post infection treatments.

Evaluation of novel fungicides under nursery and orchard conditions

Ten novel and four commonly used fungicides which found effective under *in vitro* conditions were further evaluated under nursery and orchard. The fungicides were added @ 5 l/m^2 under nursery conditions during April, June and August at the concentration mentioned in table 15. Similarly these fungicides were drenched in soil @10 l/1.5ft area around the stem in the infected plants in an orchard. The data indicated that soil drenching with cymoxanil + mancozeb (Curzate) at 0.3% was

Table 14. Effect of combined application of biocontrol agents and effective fungicides against collar rot (*P. cactorum*) disease in apple under field conditions

Treatment	Concentration (%)	Disease control (%) in treatment added before/ after planting of apple seedlings			
		20 days before	10 days before	0 days**	10 days after
Trichoderma viride-5 (TV 5)	150 g bran culture + 50 g talc based culture=A*	75.2	65.1	54.6	38.5
Bacillus sp.-11 (BS-11)	150 g coconut coir culture + 50g talc based culture= B#	75.1	71.5	64.8	50.3
Metalaxyl MZ	0.25	69.2	61.5	53.3	40.8
Metalaxyl MZ	0.3	73.4	70.3	59.1	44.9
TV5 + Metalaxyl MZ	A + 0.25	81.4	71.2	60.8	44.9
TV5 + Metalaxyl MZ	A + 0.3	89.2	75.3	64.6	54.7
BS11 + Metalaxyl MZ	B + 0.25	79.5	70.1	61.5	52.4
BS11 + Metalaxyl MZ	B + 0.3	86.7	80.4	71.8	59.7
TV5 + BS11 + Metalaxyl MZ	A + B + 0.25	85.6	77.6	72.1	63.2
TV5 + BS11 + Metalaxyl MZ	A + B + 0.3	96.6	88.4	79.9	73.3
TV 5 + BS 11	A + B	81.2	70.8	61.8	58.4
Control	-	1.4	0.3	1.2	0.8
CD at 5%	-	3.61	3.34	2.96	3.54

* TV 5 cfu/g = 2.5 x 10^6 and 2.0 x 10^3 in talc and bran based cultures, respectively
\# BS11 cfu/g = 9.1 x 10^9 each in talc and coconut coir based cultures
** target pathogen and treatments were added simultaneously

highly effective both under nursery (96.4PDC) and orchard conditions (increased shoot length 36.8 cm against 5.9 cm in control) followed by pyraclostrobin + metiram (Cabrio Top) at 0.25%, fenamidone + mancozeb (Sectin) at 0.25%, mefonoxam + mancozeb (Ridomil Gold) at 0.3% and metalaxyl + mancozeb (Matco) at 0.3%. Other fungicides *viz*., metiram, mancozeb, copper hydroxide and copper oxychloride were less effective both under nursery and orchard conditions.

Integrated management studies

The effective bio-control agents (BCAs) of fungal (TV5 and TH 15) and bacterial [BS11, *Enterobacter aerogenes*-2 (EA-2), *Pseudomonas* sp.-6 (KB6)] origin, compatible with effective fungicides *viz*., metalaxyl MZ and mancozeb, bio-resources (mustard cake, dried leaves of *Vitex negundo*, seeds of *Melia azedarach*), bio-fumigation with mustard plants, botanicals (*V. negundo*, seeds of *M. azedarach*), cow urine decoction of *Vitex* leaves + *Melia* seeds + walnut leaves and fungicides (metalaxyl MZ and mancozeb), soil solarization (nursery conditions) and approach grafting (orchard condition) were further evaluated in different combinations under nursery (sick plot) and orchard conditions to develop an integrated disease management (IDM) strategy.

In nursery, different combinations of above inputs were added 20, 10 days before, simultaneous and 10 days after planting of apple seedlings. The effective treatments were further evaluated in different combinations with soil solarization (SS). Data on seedling mortality was recorded periodically with the first appearance of disease in the month of May onwards. Final data was recorded by uprooting plants showing collar rot symptoms in the month of December using a 0-5 disease rating scale. The results indicated that pre-planting treatments were more effective in higher disease control. Further it was observed that combination of BCAs (TV5/TH15 + BS11/EA2) or amendments

Table 15. Evaluation of fungicides against collar rot under nursery and orchard conditions

Fungicide	Concen-tration (%)	Nursery	Orchard	
		Disease control (%)	Shoot growth (cm)	Lesion recovery (%)
Curzate (cymoxanil 8 %+mancozeb 64%)	0.30	96.4	36.8	32.4
Melody Duo (iprovalicarb 5.5%+propineb 61.25%)	0.25	89.5	30.8	28.8
Cabrio Top (pyraclostrobin 5%+metiram 55%)	0.25	95.8	35.1	31.1
Sectin (fenamidone 10%+mancozeb 50%)	0.25	93.6	33.8	30.4
Matco (metalaxyl 8%+mancozeb 64%)	0.30	92.6	29.6	28.4
Acrobat 50WP (dimethomorph)	0.20	75.4	23.7	22.6
Amistar 23% (azoxystrobin)	0.15	81.3	25.2	24.1
Ergon 500SC (kresoxim methyl)	0.15	86.2	27.3	25.8
Polyram 70 WG (metiram)	0.40	71.8	20.5	15.6
Ridomil Gold 68WG (mefonoxam 4% mancozeb 64%)	0.30	93.2	33.7	31.8
Indofil M-45 (mancozeb 75 WP)	0.40	72.2	21.8	16.8
Kocide 3000 (46.1 % Copper hydroxide)	0.40	70.3	19.5	12.6
Blitox (copper oxychloride 50 WP)	0.50	68.1	16.6	10.8
Infinito 68.75%SC (flupicolide 6.25% + propamacarb hydrochloride 62.5%)	0.25	83.6	26.1	25.8
Control (untreated)	-	89.4 *	5.9 **	64.8 **
CD at 5 %	-	3.18	1.62	1.14

(mustard cake + Vitex leaf) or BCAs + mustard cake with metalaxyl MZ was most effective and provided complete control of disease when applied prior to planting of seedlings. Combined applications of TH15 + EA2 + mustard cakes + metalaxyl MZ (0.3%) was highly effective followed by TV5 + BS11 + Vitex + mancozeb (0.4%), TH15 + EA-2 + metalaxyl MZ (0.25%), TV5 + B11 + metalaxyl MZ (0.25%), mustard cake + *Vitex* leaf + metalaxyl MZ (0.25%), mustard cake + *Melia azedarach* + metalaxyl MZ (0.3%) providing almost 91.0-100 per cent control when applied 10 days pre-inoculation/ pre-planting followed by simultaneous and after 10 days of inoculation. Combining these inputs with soil solarization indicated that pre-plant treatment of soil solarized beds with different treatments significantly improved the disease control. Further, it was observed that applying BCAs *viz*., TV5, BS11, TH15, EA2 (added as 150 g bran culture/ m^2 20 days prior to planting and talc formulation (TF) 10 g/ 5 l/ m^2 after one month of planting) individually with soil solarization (SS) for two months provided enhanced disease control of 96.8, 93.6, 98.0 and 99.2 per cent, respectively. Their combination (TH15 + BS11, TV5 + BS11, TH15 + EA2, TV5 + EA2) with soil solarization provided complete control. Similarly, combined application of soil amendments (mustard cake, leaves of *V. negundo*, seeds of *M. azedarach* @ 200 g/m^2), bio-fumigation with mustard plants (mulching whole plant @ 2 kg/ m^2) with soil solarization provided 97.2 97.6, 96.2 and 100 per cent control, respectively. Different combinations of bioresources with SS provided complete control when applied prior to planting seedlings in the nursery. Planting of root stock MM111 in soil solarised plot resulted in complete control.

Twelve different combinations of above BCAs, bioresources, botanical cow urine decoction, approach grafting with fungicides were evaluated under orchard condition against apple collar rot in highly infested orchards at Summa and Chhatri. Addition of mustard cake (500 g/plant), *Vitex* leaves

(3 kg/plant), *Melia* seed (2 kg/plant), botanical cow urine decoction (7.5% 5 l/plant) and bio-fumigation with mustard plant were done in the first week of March. BCAs (200 g bran culture/ coconut coir and 50 g talc culture) and fungicides *viz.*, metalaxyl MZ (0.25, 0.3%) were added in the first week of April and last week of August. Further, approach grafting alone and in combination with cow urine decoction and fungicides were also evaluated under orchard condition. Data on change in leaf colour and shoot growth were recorded in September and November (after complete leaf fall), respectively.

Results indicated that combined application of biocontrol agents (TH15, EA2), metalaxyl MZ @ 0.3% and biofumigation (mustard) was the most effective with increased shoot growth. It was followed by TH15 + EA2 + Mustard cake + metalaxyl MZ, TV5 + BS11 + mustard cake + metalaxyl MZ and TH15 + EA2 + cow urine decoction + metalaxyl MZ. Further, under orchard condition, addition of cow urine decoction (three drenchings in a year) in approach grafted plants was highly effective.

ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

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a. *PHYTOPHTHORA* DIVERSITY

Samples were collected (feeder roots, rhizospheric soil and bark of diseased citrus plants) from Meghalaya and Nagaland. *Phytophthora* was isolated from the collected samples and total of 13 isolates were obtained. They showed variation in their colony and sporangial morphology (Fig. 21). Pathogenicity test was carried out using citrus seedlings. The seedlings were inoculated with 14 days old culture grown on Czapek's dox broth. Six isolates were confirmed to produce the specific symptom of root rot disease.

b. DIAGNOSTICS

PCR based detection of *Phytophthora* spp.

DNA was isolated from 10 *Phytophthora* isolates of Meghalaya and amplified using ITS6/ITS4 primers (Fig. 22). Similarly another six isolates were amplified with primer pairs ITS1/ITS2 and A2/I2. The products were eluted, sequenced and compared.

c. DISEASE MANAGEMENT

Isolation, identification and bio-efficacy of *Burkholderia cepacia*

A new bio-control agent (*Burkholderia cepacia*) was isolated from citrus regime and profiled using BD Phoenix-100. *In vitro* bio-efficacy test of three *B. cepacia* isolates (PBY, PB2 and PB21) against *Phytophthora* sp. was done by confrontation technique. Among the isolates, isolate PB2 was found to be efficient against *Phytophthora* sp. with maximum inhibition (45.94%).

In vitro bio-efficacy of *Trichoderma* spp. against *Phytophthora nicotianae*

Among the six *Trichoderma* spp., maximum inhibition (57.85%) was recorded in

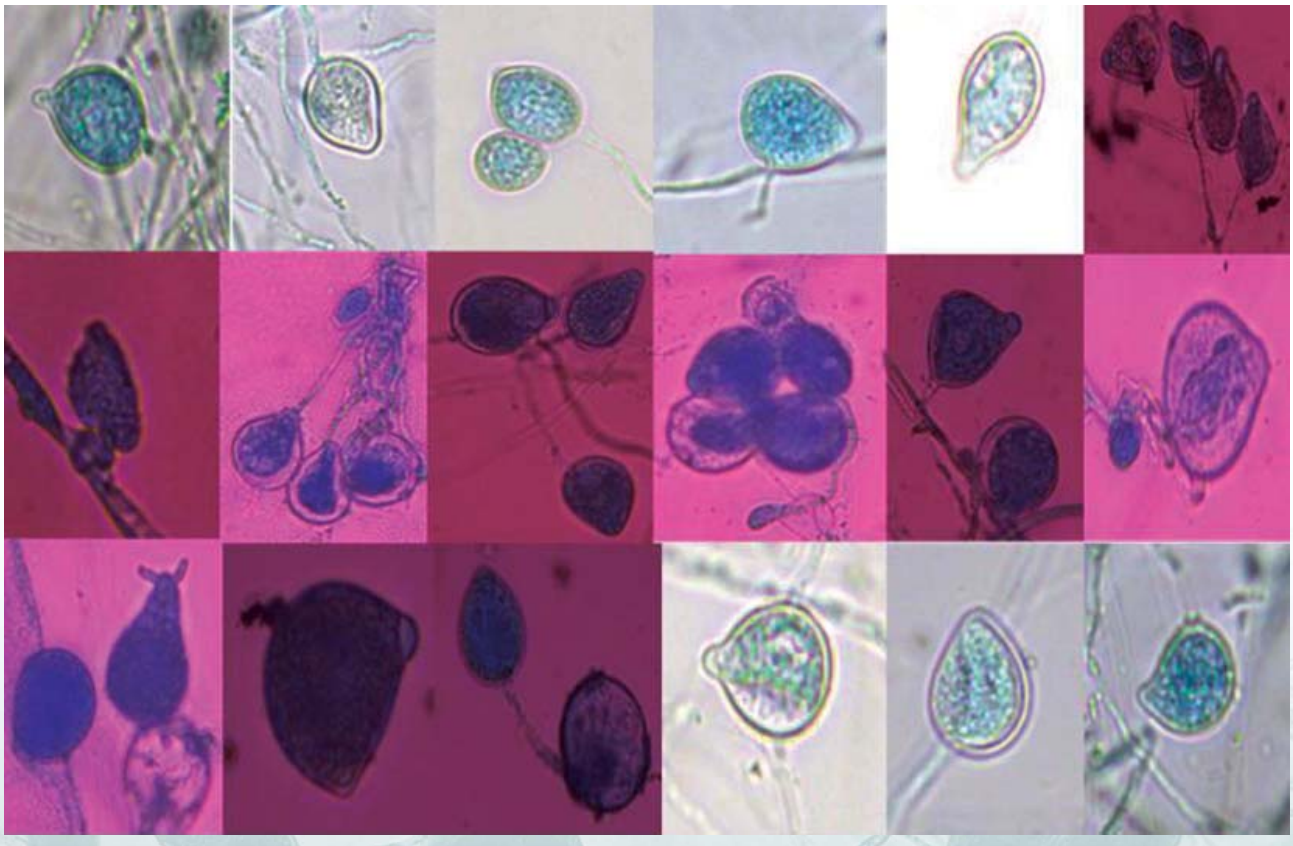

Fig. 21. Micrographs of different sporangial variation in *Phytophthora* isolates from Meghalaya and Nagaland

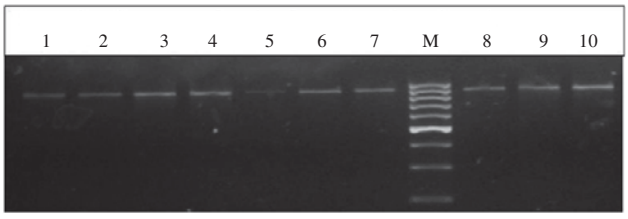

Fig. 22. PCR detection of *Phytophthora* isolates with primers ITS6/ITS4, M: 100 bp ladder, lane 1-10 represent Meghalaya isolates

T. longibrachiatum TRT followed by *T. harzianum* (56.98%), whereas the minimum inhibition was recorded in *T. brevicompactum* (46.22%) in dual culture against *P. nicotianae*.

The volatile compounds produced by these *Trichoderma* spp. varied based on the time of inoculation of antagonist and pathogen. On day '0', where both pathogen and antagonists were inoculated on the same day, there was maximum inhibition by the volatile compounds produced by *T. brevicompactum* (48.55%) followed by *T. longibrachiatum*TRT (46.51%) and *T. harzianum* (45.93%). A similar trend was observed on day 1, 2 and 3 when the pathogen was inoculated ahead of antagonists (Table 16).

The effect of non-volatile compounds produced by six *Trichoderma* spp. using 10, 20 and 30% concentration of culture filtrate indicated that *T. brevicompactum* in dual culture was found most effective (93.63%) in inhibiting *P. nicotianae* mycelium growth. At 10% culture filtrate, *T. brevicompactum* inhibited 93.44% growth followed by *T. harzianum* (53.20%) and *T. koningiopsis* (51.77%). The minimum (45.78%) inhibition was recored in *T. viride* (Table 17).

Bio-efficacy of *Trichoderma* spp. against *Phytophthora* under protected conditions

Mass multiplication of *Trichoderma* spp. was carried out in jaggery based (liquid) and bajra grain based formulations. The efficacy of these formulations in controlling root rot in citrus was tested by applying them to citrus seedlings challenge inoculated with *Phytophthora* sp. Among the two formulations, *T. harzianum* showed maximum effectiveness which was on par with Aliette (Fosetyl-Al) fungicide followed by *T. virens*.

Isolation of fungicide tolerant strains of *Trichoderma*

Trichoderma species resistant against four fungicides were isolated at different concentrations of 1, 10, 100, 1000 and 2000 ppm. The parent isolates of different *Trichoderma* spp. (*T. brevicompactum*, *T. harzianum*, *T. longibrachiatum* TRT, *T. virens*, *T. viride*, *T. asperellum* and *T. koningiopsis*) isolated from citrus soil were grown in a media containing increasing concentration of fungicides. The

Table 16. Inhibition of *Phytophthora nicotianae* by volatile compounds of *Trichoderma* spp. inoculated at different intervals

Trichoderma spp.	Inhibition (%)				Mean
	'0' day (inoculation of bio-agent and pathogen on the same day)	Bio-agent one day ahead of pathogen	Bio-agent two days ahead of pathogen	Bio-agent three days ahead of pathogen	
T. viride	43.60	44.77	47.09	49.42	46.22
T. virens	43.90	45.06	47.38	50.00	46.58
T. harzianum	45.93	47.09	49.42	51.74	48.55
T. longibrachiatum TRT	46.51	47.38	50.00	52.03	48.98
T. koningiopsis	45.06	45.93	47.97	50.29	47.31
T. brevicompactum	48.55	49.71	51.74	54.07	51.02
Mean	45.59	46.66	48.93	51.26	-
CD ($p<0.05$)	*Trichoderma* spp. = 0.746; Inoculation stage = 0.529 *Trichoderma* spp. x Inoculation stage = NS				

Table 17. Effect of non-volatile compounds produced by *Trichoderma* spp. with different concentrations against *P. nicotianae*

***Trichoderma* spp.**	**Inhibition (%)**			**Mean**
	10%	**20%**	**30%**	
T. viride	45.78	48.07	50.35	48.07
T. virens	47.49	49.78	52.35	49.87
T. harzianum	53.20	55.48	57.77	55.48
T. longibrachiatum TRT	50.06	52.35	54.63	52.35
T. koningiopsis	51.77	54.06	56.34	54.06
T. brevicompactum	93.44	93.72	93.72	93.63
Mean	56.96	58.91	60.86	-
CD ($p<0.05$)	*Trichoderma* spp. = 0.3295; Concentration = 0.2434 *Trichoderma* spp. x Concentration = 0.5691			

fungicides used were carbendazim, copper oxychloride (COC), metalaxyl + mancozeb and mancozeb. These fungicides were mixed with sterilized PDA medium to provide 1, 10, 100, 1000 and 2000 ppm. The radial growth of the mycelium was observed every 24 hours till the control plate reached full growth in each concentration. The radial growth of different species of *Trichoderma* recorded after 48 h, revealed a slow adaptation to the increasing concentration of all the tested fungicides. All the tested *Trichoderma* isolates were tolerant to COC, metalaxyl + mancozeb and mancozeb upto 2000 ppm. It was noted that the *T. viride, T. longibrachiatum* TRT, *T. virens* and *T. asperellum* behaved differently producing more growth at 2000 ppm metalaxyl + mancozeb combination than at 1000 ppm. However, no growth was recored in carbendazim amended media at concentrations above 100 ppm.

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT INSECTS, BENGALURU

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a. DISEASE MANAGEMENT

Induction of glucanase activity in cell wall glucan elicitor treated chilli plants

Thirty days-old chilli (Byadagi) plants were dipped in elicitors for 15 min/talc for 10 min and planted. There were five replications for each treatment and seven plants per replication. After two days of elicitor/talc treatment, the plants were sprayed with the sporangia of the pathogen, *P. capsici* (06-16 of IISR, Kozhikode). Leaf and stem samples collected 1, 7, 14, 21 and 28 days after inoculation were used for glucanase enzyme assays. The activity of β-1,3- and β-1,4- glucanase was determined by measuring the release of reducing sugars by using laminarin and carboxy methyl cellulose (CMC) as substrate and glucose as standard. The reduced sugar released was determined and the enzyme activity was expressed as mg of glucose released/ min/g of sample.

A significant increase in β-1,3- glucanase activity was observed in plants treated with Th9, Tv10 and Tv115 (7.4, 6.7 and 4.7 mg of glucose released/ min/g of plant tissue) which reached the highest on 14th day and declined thereafter. Treatment with Th9 elicitor resulted two fold increase in β-1,3-glucanase compared to control. In plants treated

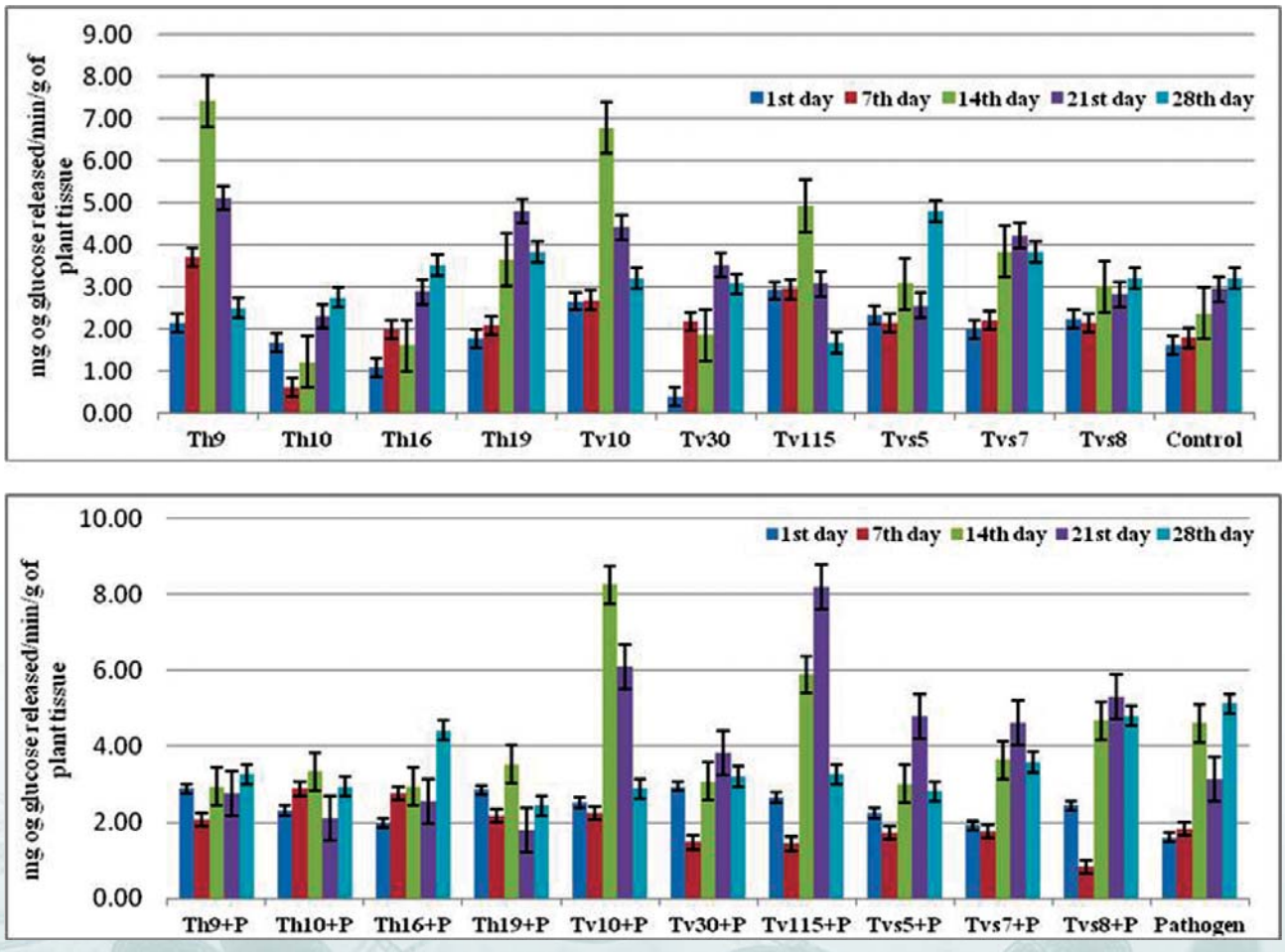

Fig. 23. β-1, 3- glucanase activity in chilli plants, (top) plants treated with elicitors only, (bottom) plants treated with elicitor and challenge inoculated with pathogen *P. capsici*

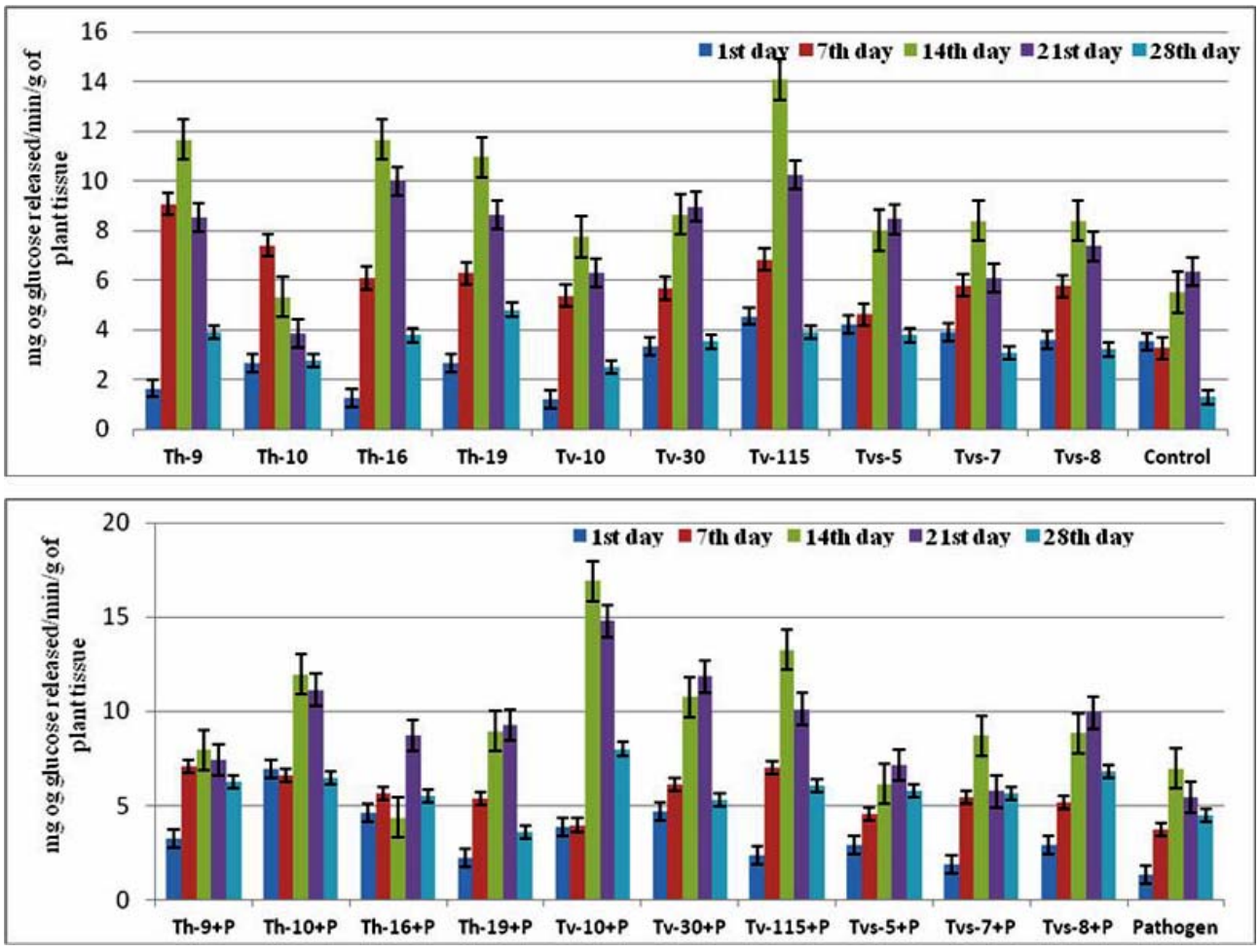

Fig. 24. β-1,4- glucanase activity in chilli plants, (top) plants treated with elicitors only, (bottom) plants treated with elicitor and challenge inoculated with pathogen *P. capsici*

with elicitor and challenge inoculated with pathogen, isolates Tv10 and Tv115 showed maximum induction of β-1,3- glucanase activity on 14th and 21st day and declined gradually compared to pathogen alone (Fig. 23). The β-1,4-glucanase activity increased on 7th day and reached the highest on 14th day which declined thereafter. Compared to control, isolate Tv-115 showed maximum induction of glucanase on 14th day (14.0 mg of glucose released/min/g of plant tissue). Treatment with Tv115 elicitor resulted in two fold increase in β-1,4- glucanase compared to control. Th10, Tv10 and Tv115 showed maximum induction of β-1,4- glucanase activity on challenge inoculation with the pathogen (Fig. 24). A similar trend was observed when talc formulations of *Trichoderma* were used.

Cloning and characterization of elicitor genes in *Trichoderma*

RNA was extracted from the mycelia of Tvs5 using the modified protocol of Chomczynski and Sacchi. c-DNA was synthesized to amplify Sm1 gene using specific Sm1 primers *viz*., SmF- (5'-GTCTCCTACGACACCGGCTA-3') and SmR (5'-GTCGAGCGCAATGTTGAA-3'). The amplified product (264 bp) was purified by gel elution and sequenced. The sequence showed 99% similarity with Sm1 gene from *T. virens*. The elicitor gene was cloned in a Chrom T-vector (pUC based T/A cloning vector). CB-5α competent cells were used for transformation and the recombinant plasmids (blue and white colony) were incubated at 37°C plates. Five white colonies were selected randomly and plasmids were isolated by alkaline lysis method. Then PCR was performed using SmF and SmR primers and sequenced using M13 forward and reverse primers. After sequencing homology search was done and showed similarity with Sm1 gene.

The PCR product was cloned into pTZ57R/T vector and the transformed colonies were observed on LB

agar plate supplemented with Ampicillin, IPTG and X-gal (Fig. 25a). Upon digestion with EcoRI and NotI double digestion, two bands were observed, one corresponding to the pTZ vector (~2886 bp) and the other corresponding to the insert (gene of interest, ~380bp) (Fig. 25b).

Morphological and molecular identification of selected isolates

Trichoderma isolates were plated on MA and incubated at 21^{0}C for 48 h in dark and 48 h in light and on SNA and PDA and incubated at 30^{0}C for 72 hours and mycelial growth was recorded. By slide culture technique the length and width of conidia and phialides, conidial shape, colour, ornamentation and presence or absence of chlamydospores were recorded. The morphological identification was confirmed using online interactive tool available at USDA. Molecular identification of selected *Trichoderma* isolates was done by amplifying ITS region (~650bp) using primers ITS1-(5'-TCTGTAGGTGAACCTGCGG-3') and ITS4-(5'-TCCTCCGCTTATTGATATGC-3') and further confirmation was done by amplifying large intron portion of *Tef1* region (~350bp) using EF1-728F(5'-CATCGAGAAGTTCGAGAAGG-3') and EF1-986R(5'-TACTTGAAGGAACCCTTACC-3') primers as described by Druzhinina *et al*. (2005). After sequencing, homology search was done by using NCBI BLAST and TrichOKEY and the sequences were submitted in GenBank.

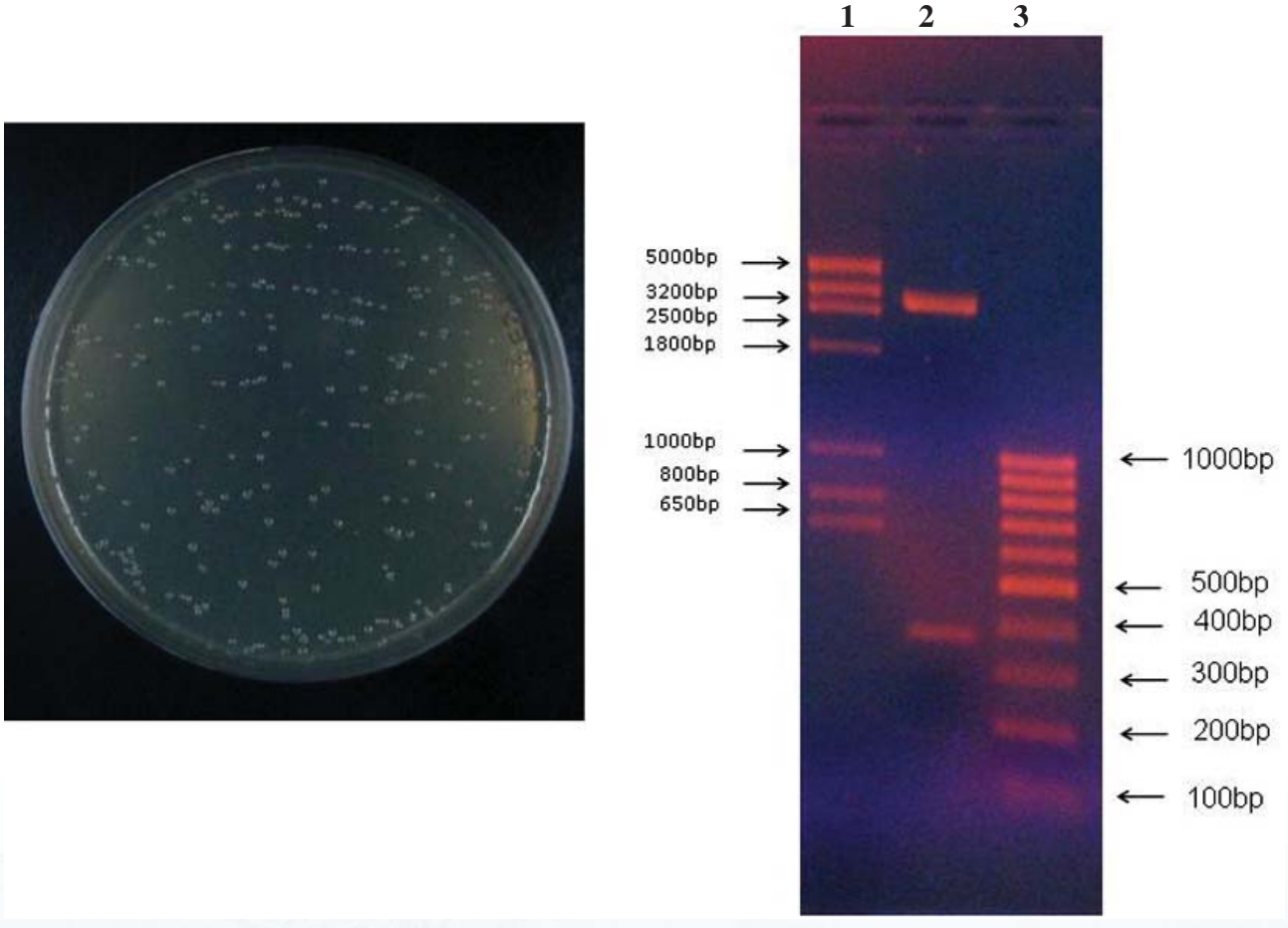

Fig. 25. Cloning of elicitor gene in *Trichoderma viride*, (left) LB ampicillin agar plates showing the colonies ligated with both insert and vector; (right) 1.5% agarose gel electrophoresis after restriction digestion of pTZ-NBAII with *Eco*RI and *Not* I, 1: DNA size marker; 2. pTZ-MBAII; 3: 100bp DNA ladder

NATIONAL RESEARCH CENTRE FOR CITRUS, NAGPUR

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Co-investigators:
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a. *PHYTOPHTHORA* DIVERSITY

Collection and conservation of *Phytophthora* spp. isolates infecting citrus

In total, 28 orchards and six nurseries in Vidarbha region of Maharashtra, Kadappa and Anantapur districts of Andhra Pradesh and Tinsukhia region of Assam were surveyed and soil, root, leaf, fruit and water samples were collected from plants infected with foot and collar rot, root rot and gummosis. Samples were then assayed on a selective medium containing pimaracin-ampicilin-rifampicin-PCNB-hymexazol (PARPH) and the propagules per cm^3 soil were determined by dilution plating. Baiting technique (using rough lemon leaf bits) was followed to isolate *Phytophthora* spp. from the soil samples which failed to produce any colony in selective medium.

During the year, 28 *Phytophthora* spp. isolates (18 isolates of *Phytophthora nicotianae*, six isolates of *P. palmivora*, three isolates of *P. boehmeriae* and one isolate of *P. citrophthora* were isolated, purified and included in our existing accessions of *Phytophthora* spp. and so far a total of 149 isolates have been isolated, purified and being maintained in sterile distilled water at room temperature ~25^{0}C. The same sets of cultures were also maintained in CMA plates by periodical subculturing in a BOD incubator at ~25^{0}C. One of our earlier collected isolates (NRCPh112, from rhizosphere soil of a citrus orchard in Sriganganagar, Rajasthan) *Phytophthora* taxon Salixsoil was re-designated as *Phytophthora lacustris* as suggested by Nechwatal *et al*. (2012). NRCPh-119 (*Phytophthora insolita*) culture was deposited at National Bureau of Agriculturally Important Microorganisms (NBAIM), Kusmaur, Mau, India (Accession no. NAIMCCF- 03051).

Diversity analysis

Colony morphology: Colony morphology was recorded as pattern, nature of margin and growth rate of isolates on V8 agar media and on PDA after four days of growth at 25$\pm$1^{0}C in the dark. 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. In case of *P. nicotianae* isolates, colonies with cottony mycelia (six isolates) and dense cottony mycelia (13 isolates) with no pattern having irregular margin were observed on V8 agar whereas more than one pattern was observed on PDA i.e. cottony mycelia with uniform margin (six isolates), dense cottony mycelia with uniform margin (nine isolates) and stoloniferous with irregular margin (four isolates).

All the *P. palmivora* isolates showed stellate striated pattern with uniform margin on V8 agar whereas less defined petalloid pattern with irregular margin was observed on PDA. *P.boehmeriae* isolates showed petalloid pattern with irregular margin on V8 agar while on PDA cottony mycelia with no pattern and uniform margin was observed. The only *P. citrophthora* isolate showed colony with stellate pattern with irregular margin was observed on V8 agar while floral/ pettaloid pattern was observed on PDA. The colony morphology of six different *Phytophthora* spp. (*Phytophthora nicotianae*, *P. palmivora*, *P. citrophthora, P. boehmeriae, P. insolita* and *P. lacustris*) isolated from citrus tissues/ rhizosphere, on V8 agar and PDA is illustrated in Fig. 26 and 27. The growth rate varied from 6.25 – 17.12 mm/ day in V8 agar and 3.87 – 10.62 mm/ day in PDA for above isolates.

Sporangial morphology: Sporangial morphology was checked through agar-disk-in-water technique. In *P. nicotianae* isolates sporangia varied from ovoid to globose with prominent papillae and non-caducous. Sporangiophore showed a simple sympodial branching. Sporangial LXB range was 28.57-48.5 µm x 20.82-38.8 µm with an average of 40.25 µm x 30.9 µm whereas length: breadth ratio ranged from 1.19-1.46 with an average of 1.29. In case of *P. palmivora*, sporangia are variable in shape, mostly ovoid to globose, ellipsoid, limoniform with prominent papillae and caducous. Sporangiophore showed sympodial and simple sympodial branching. Sporangial LxB range was 43.76-55.7 µm x 31.61-38.61 µm with an average of 44.11 µm x 32.68 µm whereas length: breadth ratio range was 1.26-1.53 with an average of 1.34.

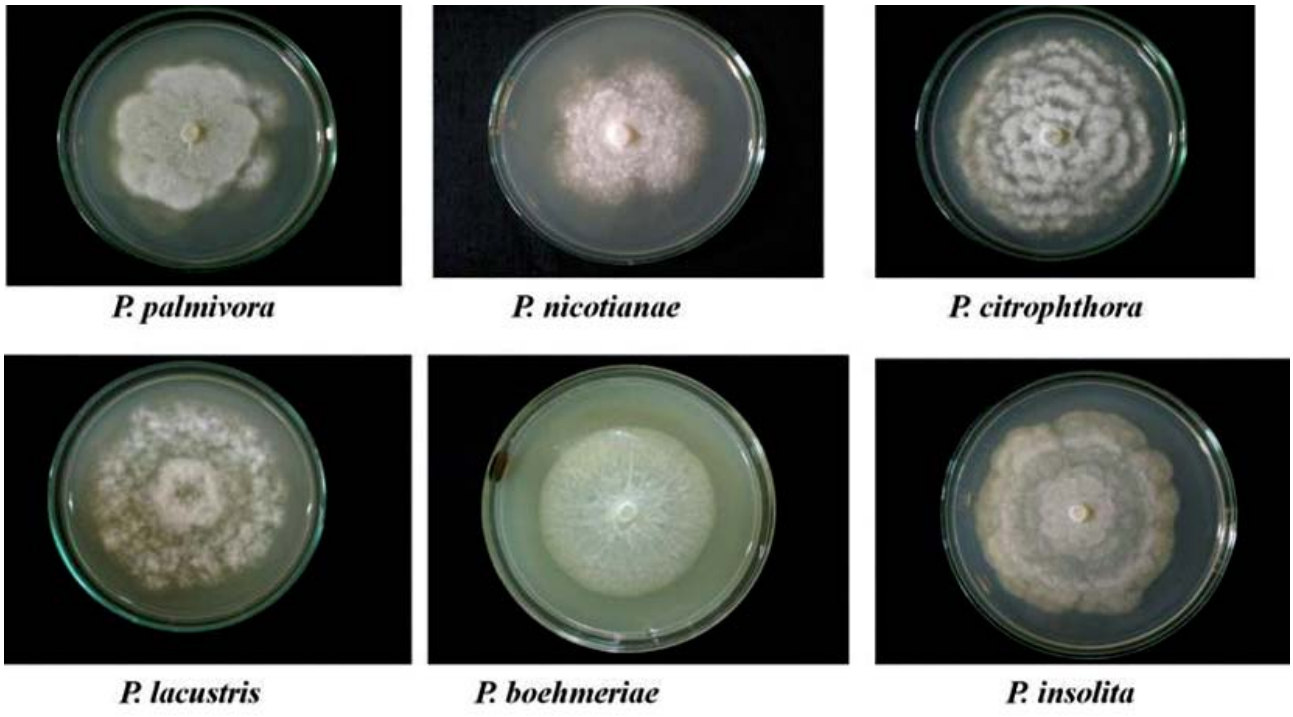


Fig. 26. Colony morphology of *Phytophthora* isolates from citrus cultured on V8 agar

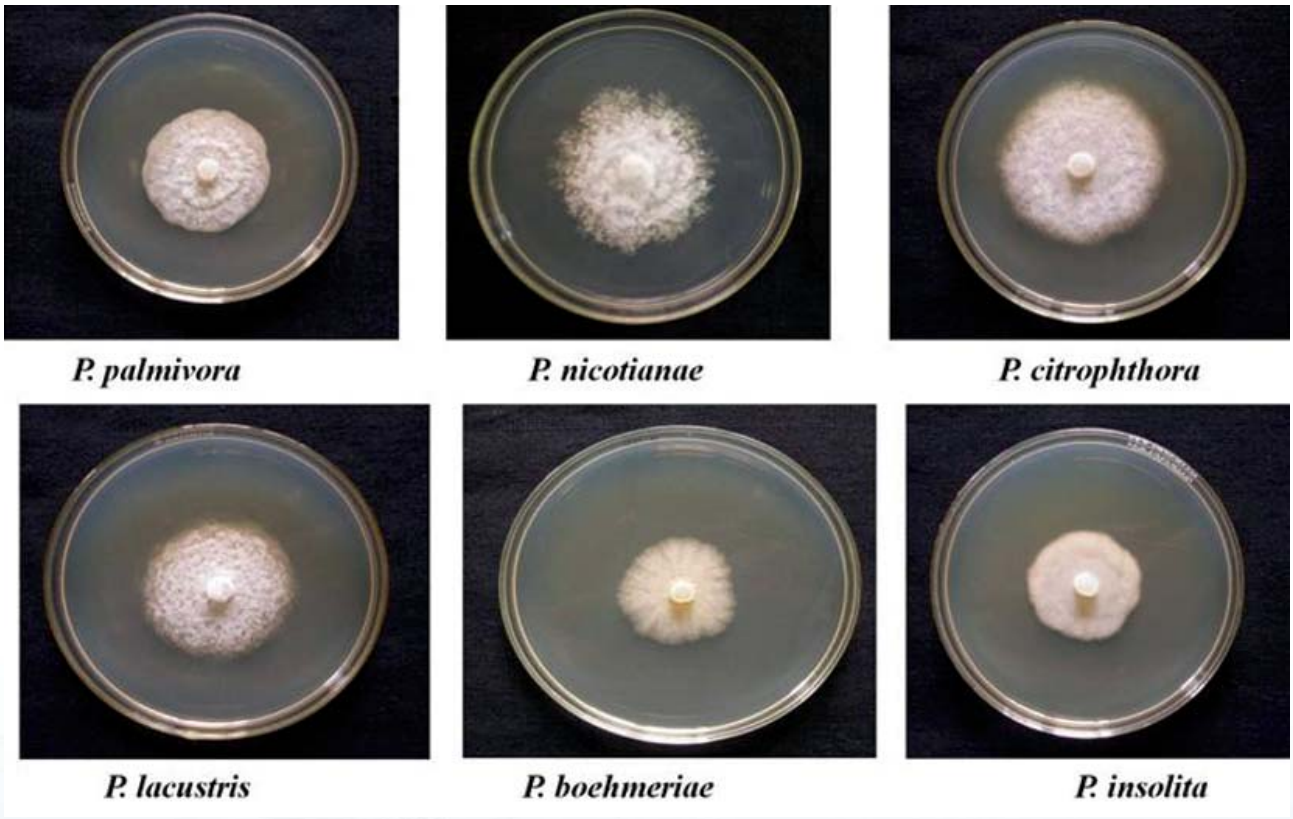


Fig. 27. Colony morphology of *Phytophthora* isolates from citrus cultured on PDA

P. citrophthora sporangiophores are irregularly branched, some singly and some in a loose sympodium. Sporangia were globose to ovoid with papillae and non caducous. Sporangial LxB range was 44.9-29.99 µm x 27.76-37.51 µm with average of 37.1 µm x 31.76 µm whereas length: breadth ratio range was 1.08-1.25 with an average of 1.16. *P. boehmeriae* sporangia were globose with distinct papilla and caducous with a sporangial LxB of 33.95 x 29.1 µm (Fig. 28).

Mating type: The mating types of the collected isolates (NRCPh 120-149) were detected in crosses

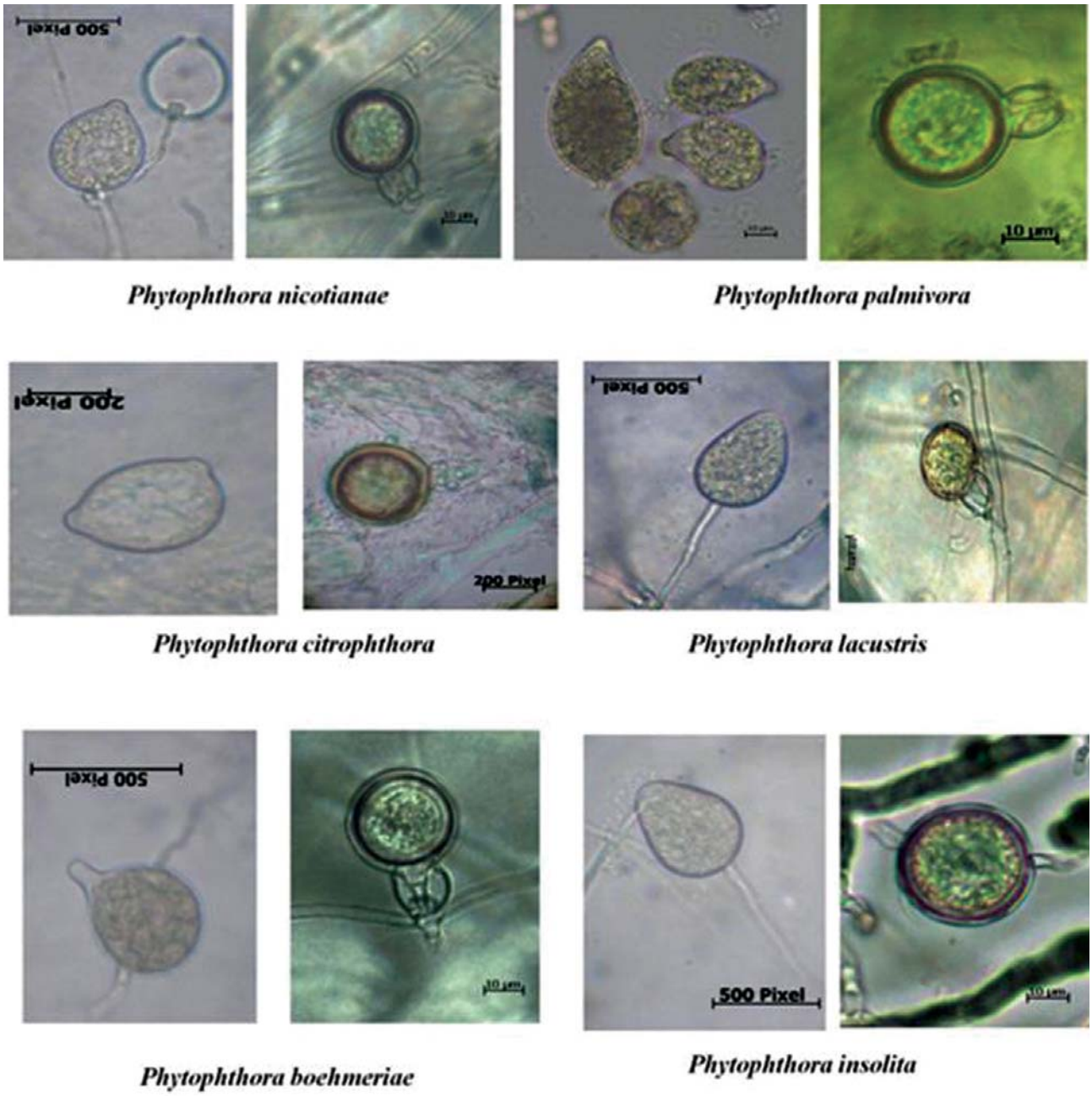


Fig. 28. Sporangial and oogonial morphology of six species of *Phytophthora*

between the known mating type (*P. nicotianae*) with unknown isolated samples on carrot agar medium incubated at 20^{0}C at dark for 3-4 days to develop oospores. Out of the 30 isolates, only one isolate (NRCPh -123, *P. nicotianae*) was found as A2 mating type. The oospore formed was round 21.53 mm in diameter. Rest 26 isolates were found as A1 mating type. All *P. boehmeriae* isolates were found homothallic. In *P. nicotianae* isolates, oogonial diameter range was 22.28-29.22 µm with an average value of 25.38 µm whereas oospore diameter range was 14.9-24.24 µm with an average value of 21.15 µm. Antheridia were amphigynous with LxB range of 9.43-13.2 µm x 10.87-14.27 µm with an average of 11.53 µm x 12.74µm.

Metalaxyl sensitivity: Fungicide mefenoxam sensitivity was determined by growing the isolates on corn meal agar (CMA) amended with mefenoxam-mancozeb. Mycelial agar discs of size 5 mm were placed on plates of different concentrations and incubated in the dark at 25±1°C. The radius of the mycelial growth was recorded when the circumference of non amended media plate was completely covered by mycelial growth. An isolate was scored as sensitive (S) if colony growth on media amended with 5 µg ml^{-1} mefenoxam-MZ was less than 40% of the isolate growth on non amended media. Moderate isolates (M) exhibited growth on media amended with 5 µg ml^{-1} greater than 40% of that on non amended

media. There was no resistant isolate (R) that exhibited growth on media amended with 50 µg ml^{-1} greater than 40% of that on non-amended media (rather complete inhibition of growth on media amended with 50 µg ml^{-1} of mefenoxam-MZ was observed). Out of the 20 (NRCPh 101 – NRCPh 120) isolates, only one isolate (NRCPh 112) was found moderately resistant to mefenoxam-MZ, rest all were found sensitive.

Molecular diversity analysis: Total genomic DNA from approx. 100 mg of ground mycelium was extracted from 30 *Phytophthora* spp. isolates (Acc no. NRCPh 120-149). PCR amplification of the ITS region of the template DNA was performed using the primers ITS4 and ITS6. The PCR amplicons obtained from all the isolates were of expected size (~900 bp). The PCR product was digested with the restriction enzymes *Msp*I and *Alu*I, according to the enzymes manufacturer's instructions and the restricted products were electrophoresed in 2.5% agarose gel and documented. The results of RFLP analysis of the PCR products of all the isolates under study revealed a difference in their restriction pattern. *Msp*I digestions revealed three bands of 404, 390 (seen as one broad band of 400 bp) and 120 bp in *P. nicotianae* isolates whereas two clear bands of 508 and 389 bp were observed in *P. palmivora* isolates. Correspondingly, *Alu*I digestion revealed three bands of 745, 117 and 52 bp in *P. nicotianae* isolates, while restriction fragments of 501, 160, 157 and 42 bp were noticed in *P. palmivora* isolates. In case of *P. boehmeriae* isolates, *Msp*I digestions revealed two bands of 550 and 360 bp. This shows close confirmity with the morphological analysis.

ITS RFLP profile of six *Phytophthora* species isolated from citrus tissues/ rhizosphere

In a robust PCR-based assay involving the amplification of a ~900 bp amplicon and its

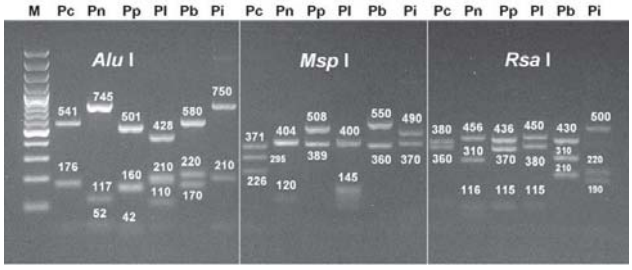


Fig. 29. ITS-RFLP profiles of six different species of *Phytophthora* from citrus obtained after digestion with *Alu*I, *Msp*I, and *Rsa*I. The fragment sizes are given in base pairs above each band. M – 100 bp marker, Pc - *Phytophthora citrophthora*, Pn – *P. nicotianae*, Pp – *P. palmivora*, Pl - *P. lacustris*, Pb – *P. boehmeriae* and Pi – *P. inosita*

digestion with three restriction enzymes (*Msp*I, *Alu*I and *Rsa*I), a characteristic profile or fingerprint was produced for six *Phytophthora* spp. (*Phytophthora nicotianae*, *P. palmivora*, *P. citrophthora, P. boehmeriae, P. insolita* and *P. lacustris*) isolated from citrus tissues/ rhizosphere. The band sizes are shown in table 18 and fig. 29. This ITS-RFLP data would be of immense help for rapid and accurate identification/ diagnostic tool of the above six *Phytophthora* species and will assist in the development and enforcement of phytosanitary legislation for the *Phytophthora*-induced diseases.

Multi-locus sequencing: ITS sequences of 43 isolates of *Phytophthora* spp. were submitted in the GenBank databases. When these sequences were compared with the known *Phytophthora* species available in the GenBank, > 96% homology (at the ITS 1, 5.8S and ITS 2 regions of the nuclear ribosomal DNA) was observed with respective *Phytophthora* isolates.

Amplification of additional loci /genes was carried out for the following regions: *β-tubulin,* elongation factor-1β (*EF-1β*) gene region, cytochrome oxidase-1 (*COX-1*) gene and cytochrome oxidase-2 (*COX-II*) gene using the previously standardized

Table 18. Fragment sizes (base pairs) of the internal transcribed spacer regions of six *Phytophthora* species after digestion with restriction enzymes *Msp*I, *Alu*I and *Rsa*I

Species	*Msp*I	*Alu*I	*Rsa*I
P. nicotianae	404, 390, 120	745, 117, 52	456, 310, 116
P. palmivora	508, 389	501, 160, 157, 42	436, 370, 115
P. citrophthora	371, 295, 226	541, 176, 175	380, 360
P. insolita	490, 370	750, 210	500, 220, 190
P. boehmeriae	550, 360	580, 220, 170	430, 310, 210
P. lacustris	400, 145	428, 210, 110	450, 380, 115

PCR conditions and primers. Some of the selected amplified PCR products with respect to above genes were sequenced and submitted to NCBI GenBank database.

Phylogenetic analysis of ITS sequences: A total 28 ITS region sequences of *P. nicotianae* isolates from various countries were compared for diversity using maximum parsimony (1000 bootstrap) method (Tamura *et al*. 2007). The results showed that the ITS sequences of *P. nicotianae* isolates were highly conserved regardless of geographical origin. In the phylogenetic tree (Fig. 30), *P. nicotianae* isolates originated from citrus orchards of Vidarbha and other parts of the world (USA, Taiwan, Japan, Cuba, Turkey, Thailand) clustered in one clade irrespective of the geographical origin. The isolates NRCPh 5 and 6 formed a separate group in the clade with moderately high bootstrap value indicating intra-specific sequence variation among Vidarbha isolates. Interestingly, one *P. nicotianae* isolate infecting betelvine in India (GenBank accession no. FN263242) form a different group from the rest of the isolates.

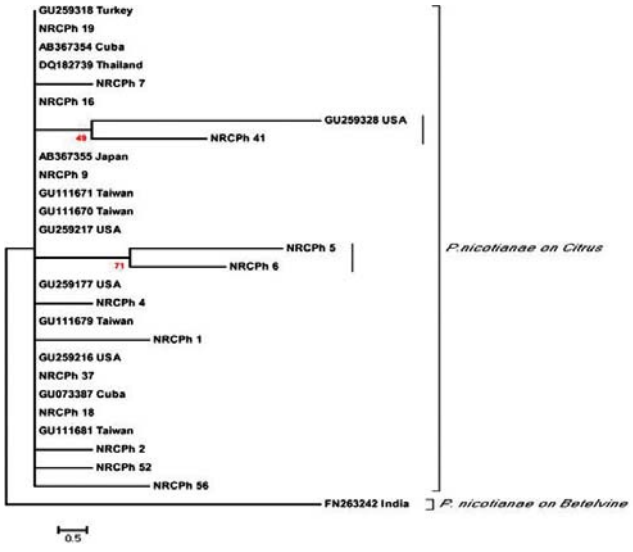


Fig. 30. Maximum parsimony analysis of ITS sequences of *Phytophthora nicotianae* isolates

Diversity studies using SSR markers: Amplification of SSR loci from *P. palmivora* and *P. nicotianae* isolates was performed using 10 SSR primer pairs (Schena *et al.* 2008). Out of 10 SSR primer pairs one pair (S8F and S9R) showed specific banding patterns linked with species. In that three prominent bands of size 650 bp, 1 kb and 1.8 kb were found to be linked with *P. palmivora* whereas 900 bp, 2 kb and 2.5 kb size bands were linked with *P. nicotianae* mediating differentiation between two *Phytophthora* species. The reproducibility of this SSR primer pair confirmed the robustness of this technique.

b. DIAGNOSTICS

Detection of *Phytophthora palmivora* using species-specific primer pairs

A *P. palmivora*-specific primer pair (Pal1s: CAC GTG AAC CGT ATC AAA ACT and Pal2a: CAA TCA TAC CAC CAC AGC TGA) was designed from the sequences encompassing the ITS1-5.8S rRNA-ITS2 regions as reported by Tsai *et al.* (2006). The specificity of these two primers was verified by PCR using DNA prepared from four *Phytophthora* spp., which represented species isolated from diseased citrus plants collected from different areas of the country. The primer set Pal1s D Pal2a was able to amplify a unique DNA fragment of 648 bp in *P. palmivora* samples.

Detection of *Phytophthora* spp. in gummosis-infected tissues

Bark tissues were collected from Nagpur mandarin plants showing symptoms of mild to moderate gummosis. All infected bark samples were washed with running tap water, dried with blotting paper, cut into segments and ground in liquid nitrogen with a mortar and pestle. DNA was extracted from100 mg of powdered tissue and quantified. PCR amplification of ribosomal ITS regions was performed with the primers ITS4 and DC6, to specifically amplify ribosomal DNA from the major pathogenic oomycete groups. PCR products from first reaction were then amplified in a second, semi-nested round, using universal primers ITS4 and ITS6. Ten µl of the amplification products generated with primer pair ITS4 and ITS6 were digested with restriction enzyme *Msp*I in a total volume of 20 µl according to the manufacturer's instructions and the amplification was analyzed in 2.5% agarose gels.

The primer pair DC6/ITS4 produced 1.2 kb fragment and an amplification product of ~ 900 bp, typical for the genus *Phytophthora* was obtained after nested PCR with primers ITS4 and ITS6. *Msp*I digestions revealed two clear bands of 508 and 389 bp indicating presence of *P. palmivora* in all nine diagnostic samples (Fig. 31). *P. nicotianae* specific 120 bp band also appeared in some samples. The species-specific primer pairs NIC1/ NIC2 and Pal1s D Pal2a were also investigated for diagnosis of *P. nicotianae* and *P. palmivora,* respectively (Fig. 31).

Real-Time PCR (TaqMan) detection of *P. nicotianae*

DNA from fungal mycelia collected from soil, root and water were used in the study. The following

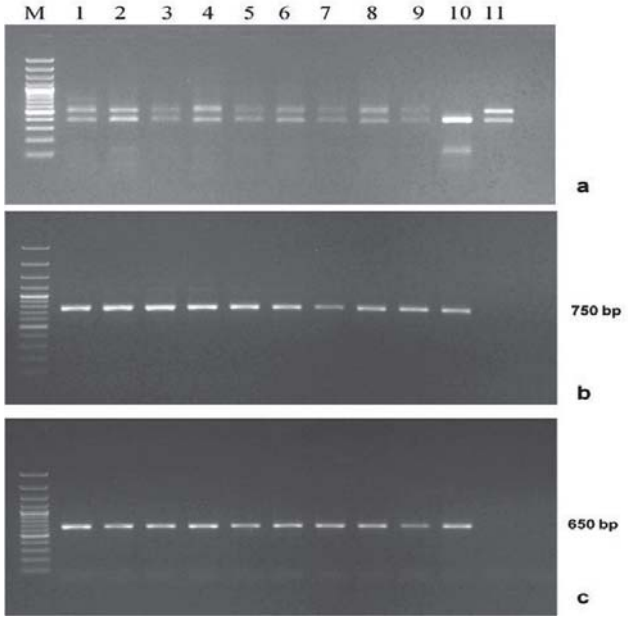


Fig. 31. Molecular identification of *Phytophthora* spp. in gummosis infected citrus barks through PCR-RFLP and species specific primers. (a) Restriction profile with *Msp*I, (b) Amplicons obtained using primers Nic1/Nic2, (c) Amplicons obtained using primers Pal1s D Pal2a. M – 100 bp marker, Lane 1-9 – Samples from gummosis affected citrus trees

primers and probe (Taqman) were designed from the ITS region of *P. nicotianae* as reported by Huang *et al*. (2009).

P.nicF: 5' -GAA CAA TGC AAC TTA TTG GAC GTT T- 3'

P.nicR: 5' –AAC CGA AGC TGC CAC CCT AC– 3'

Pn.Pro: 5' –/56-FAM/TTC ACC AGT CCA TCA CGC CAC AGC/6TAMARA/– 3'

The real-time PCR amplifications were performed with ABI 7300 (Applied Biosystems, Foster City, CA) machine. The sensitivity of the primers was assessed using serial dilutions of total DNA extracted from *P. nicotianae*. In the 10-fold dilutions, series of *P. nicotianae* DNA extracted from pure cultures, the detection limit was found 5.8 ng D µl - 58 fg D µl (Fig. 32). Standard curves showed a linear correlation between input DNA and Ct after the TaqMan PCR (Fig. 33). The correlation coefficient (r^2) of the standard curve was 0.987. *P. nicotianae* was also detected in soil, root and water samples collected earlier from citrus orchards. Ct values are mentioned in table 19.

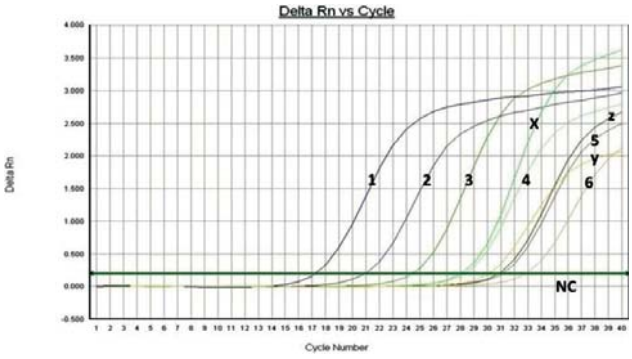


Fig. 32. Sensitivity of TaqMan PCR assay assessed by 10-fold serial dilutions of Phytophthora nicotianae DNA extracted from pure culture alongwith some diagnostic samples. Real time amplification curve of different concentrations of DNA: 1-6: 10 fold dilution of P. nicotianae DNA (5.8 ng/µl – 58 fg/µl), x = soil DNA, y = root DNA, z = water DNA, NC: PCR negative control containing no DNA.

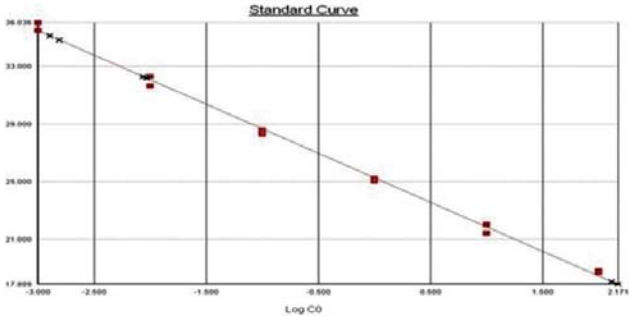


Fig. 33. Standard curve showing the relationship between copy number and corresponding Ct value

Table 19. Ct values for different samples analyzed through Real-time PCR method

Sample	Ct	Standard deviation-Ct
P. nic1	17.22	0.227
P. nic2	20.95	0.211
P. nic3	24.38	0.292
P. nic4	28.26	0.048
P. nic5	31.11	0.217
P. nic6	32.87	0.035
Soil	28.05	0.601
Root	30.07	0.032
Water	30.82	0.017

P. nic1-6 are 10-fold dilutions of *Phytophthora nicotianae* DNA

c. HOST RESISTANCE

Screening of citrus rootstocks against *Phytophthora* spp.

Fourteen different rootstock seedlings *viz*., Rough Lemon-308, Rough Lemon-340, Rough Lemon-315, Rough Lemon-349, Rough lemon-322, Rough Lemon-62, Rangpur Lime-307, Rough Lemon IPS-77, Rough Lemon-325, Rough lemon IPS-85, Jatti Khatti, Karna-337, *Citrus karna*, and Rangpur Lime-313 were inoculated with *P. nicotianae* (chlamydospores and freshly released zoospores). under glasshouse conditions After six weeks of inoculation, the seedlings were uprooted by carefully removing the pot mix with minimum disturbance to roots. Observations on root rot and *Phytophthora* population were recorded. Results (Table 20) indicated that all the rootstocks were susceptible to *P. nicotianae* root rot. However, the rootstock Rough lemon 325 was found moderately tolerant whereas rest all were found susceptible to highly susceptible.

d. DISEASE MANAGEMENT

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

Three fungal and four bacterial bioagents isolated and purified recently were maintained for studying their antagonistic activities against *Phytophthora* spp. infecting citrus.

Multilocational testing of *Trichoderma* isolates obtained from PhytoFuRa centres

In vivo efficacy studies: Fifteen *Trichoderma* isolates (PhytoFuRa 1 – 15), obtained from different PhytoFuRa nodal centers were selected for *in vivo* efficacy studies of antagonism against *Phytophthora.* Six- month- old seedlings of rough lemon were raised in polybags and inoculated with 1 % (w/v) culture of bioagents (*Trichoderma* spp.) mixed with the potting mixture. After one month of establishment of the inoculum, 25-ml zoospore suspension of *P. nicotianae* were added to each polybag. The initial population of *Phytophthora*

Table 20. Screening of citrus rootstocks against *P. nicotianae* root rot

Rootstock	Reduction in root length (%)	Reduction in root wt (%)	Root rot rating[b]	*Phytophthora* /cc soil after 6 weeks
Rough lemon-308	30.6 (33.6)	47.7 (43.7)	3.2	383
Rough lemon-340	24.0 (29.3)	50.7 (45.4)	2.8	258
Rough lemon-315	4.6 (12.4)	36.4 (37.1)	3.4	278
Rough lemon-349	10.9 (19.3)	50.4 (45.2)	2.8	195
Rough lemon-322	28.1 (32.0)	63.2 (52.6)	3.8	182
Rough lemon-62	18.6 (25.5)	31.3 (34.0)	2.6	517
Rangpur lime-307	17.8 (24.9)	51.9 (46.1)	3.6	207
Rough lemon IPS-77	29.9 (33.1)	57.4 (49.3)	2.6	522
Rough lemon-325	10.9 (19.2)	48.9 (44.4)	1.8	188
Rough lemon IPS-85	20.6 (27.0)	48.0 (43.8)	2.6	155
Jatti Khatti	18.5 (25.5)	45.6 (42.5)	3.0	310
Karna-337	17.3 (24.6)	37.9 (38.0)	2.8	302
Citrus karna	15.7 (23.3)	50.9 (45.5)	3.6	213
Rangpur lime-313	13.1 (21.2)	8.6 (16.0)	3.4	307
CD (P = 0.05)	3.9	5.2	1.1	

[a]Figures in parentheses are angular transformed values

[b] Root rot rating scale (modified after Grimm and Hutchison, 1973), Scale of 1 – 5 : 1 = No visible symptoms, 2 = A few roots with symptoms (1-25 % rotted), 3 = Majority of roots with symptoms (26 – 50% rotted), 4 = All roots infected, cortex sloughed from major roots (51-75 % rotted), and 5 = majority roots dead or missing (> 76% rotted)

was assessed to establish the propagule density 20-30 cc pot mix. The population of bioagents was maintained throughout the experiment at 10^6 in rough lemon seedlings. After four months, the treated seedlings were uprooted. The roots were washed and screened for total root length and rotted roots by using a root scanner (Biovis, Mumbai). Results indicated that all the treatments were able to reduce root rot up to a certain amount. However, maximum reduction was observed with the isolate PF-6 (root rot rating 1.7). The same treatment was also found to induce maximum root and shoot growth (Table 21). PF-5 and PF-11 were also found effective in lowering root rot intensity.

Evaluation on the efficacy of NRCfBA-44, a promising strain of *T. harzianum* in reducing the population of *Phytophthora* in naturally infested rough lemon rootstock seedlings was done in a private nursery. The seedlings were inoculated with the bioagent after multiplying it on sorghum grains. Results indicated that *Phytophthora* population decreased from 447 to 95 (a reduction of 78.7%) after inoculation with NRCfBA-44 while in control plants *Phytophthora* population increased from 190 to 443 (an increase of 133%). This confirms the efficacy of NRCfBA-44 in reducing the population of *Phytophthora* in nursery planting materials. Morphological, cultural and molecular characterizations have been done for the two most promising isolates of *T. harzianum* (NRCfBA – 29 and NRCfBA – 44).

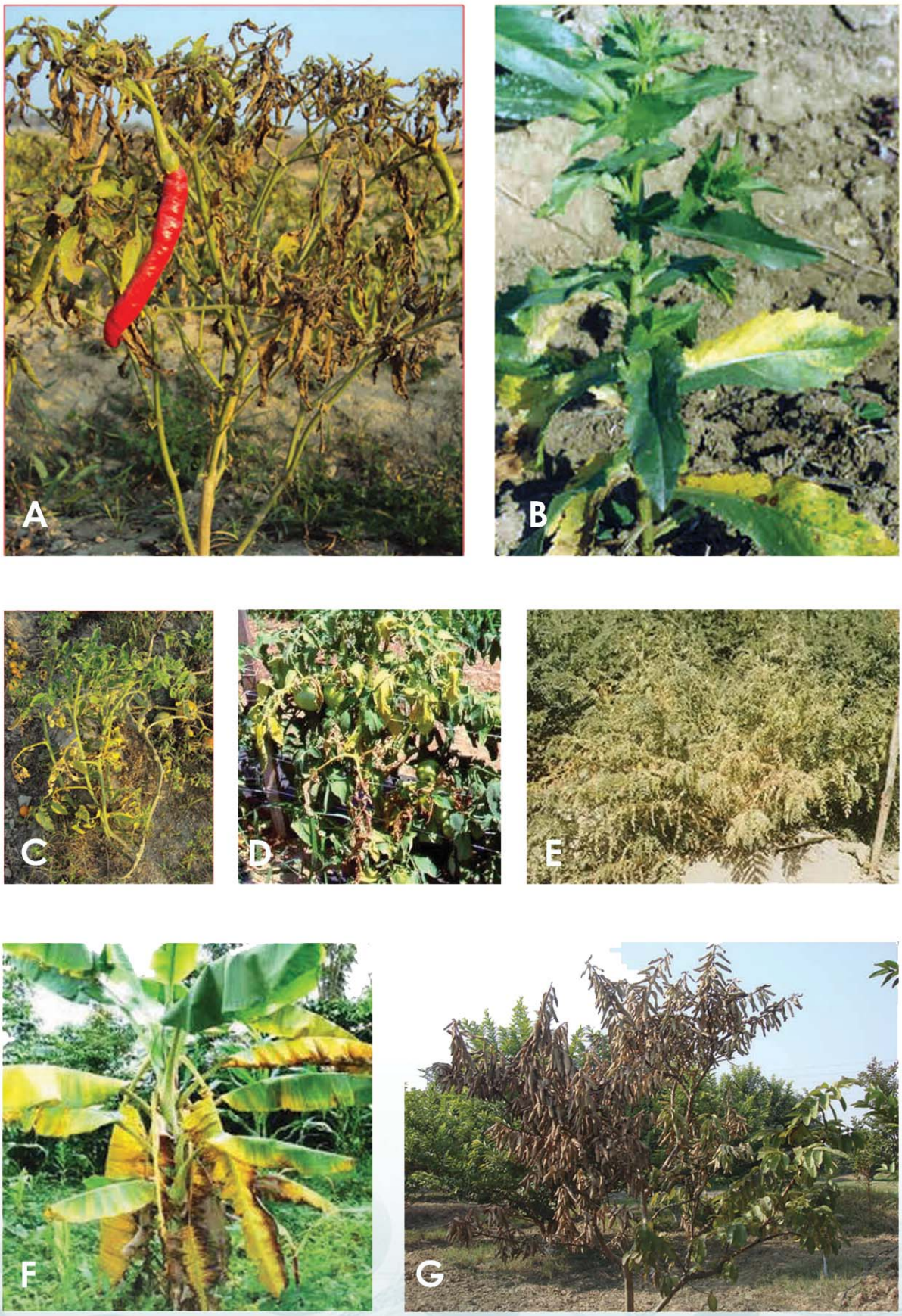
Table 21. Effect of *Trichoderma* isolates (Phytofura 1 – 15) on growth of rough lemon seedlings and root rot due to *P. nicotianae*

Treatment	Root length (cm)	Average root rot rating [a]	Dry wt of root (g)	Dry wt of shoot (g)	Total shoot length (cm)	Bioagent population initial x 10^6	Bioagent population at end x 10^5	*Phytophthora* population Initial / cc soil	*Phytophthra* population at end / cc soil
PF-1	126.8	3.6	1.88	3.74	47.85	0.6	1.6	274	60
PF-2	112.6	3.8	1.14	2.64	45.12	7.8	1.6	413	65
PF-3	117.1	3.5	1.36	2.99	50.56	12.4	2.0	395	40
PF-4	117.7	3.8	1.37	3.02	40.04	4.6	0.8	325	65
PF-5	137.5	2.3	2.27	4.03	52.25	13.8	2.0	202	63
PF-6	149.2	1.7	2.68	4.65	56.51	8.2	1.2	290	18
PF-7	126.5	3.5	1.76	3.25	48.37	0.6	0.8	265	97
PF-8	130.7	3.2	1.63	3.64	45.25	8.8	2.4	274	52
PF-9	123.5	3.7	1.59	3.06	42.62	4.8	2.4	248	32
PF-10	131.3	3.5	1.58	3.66	50.10	8.0	2.8	234	60
PF-11	136.1	2.4	2.14	4.05	52.37	11.4	1.6	167	45
PF-12	123.5	3.5	1.77	3.24	46.57	7.6	1.2	294	78
PF-13	136.2	3.5	1.99	3.80	52.64	8.2	0.8	230	65
PF-14	128.5	3.6	1.79	3.24	46.5 2	5.6	0.8	340	75
PF-15	129.8	3.7	1.36	3.25	47.62	4.5	1.6	354	77
Healthy control	139.5	1.0	2.56	3.88	47.37	-	-	-	-
Control infested	43.5	4.8	1.13	2.63	28.12	-	-	286	147
CD (P=0.05)	6.8	0.52	0.34	0.82	4.7				

a Root rot rating Scale (modified after Grimm and Hutchison, 1973), Scale of 1 – 5 : 1 = No visible symptoms, 2 = A few roots with symptoms (1-25 % rotted), 3 = Majority of roots with symptoms (26 – 50% rotted), 4 = All roots infected, cortex sloughed from major roots (51-75 % rotted), and 5 = majority roots dead or missing (> 76% rotted)

PhytoFuRa

Fusarium



Fusarium wilt of A. Chilli, B. Safflower, C. Tomato, D. Guava,
E. Banana, F. Chick pea, G. Pigeon pea

CENTRAL INSTITUTE FOR SUBTROPICAL HORTICULTURE, LUCKNOW

Principal investigator:
Dr. B. K. Pandey

Co-investigator:
Dr. M. Muthu Kumar

a. *FUSARIUM* DIVERSITY

Enumeration of total microbial population from different guava orchards

Soil samples of guava orchards from different locations were collected before the onset of rains to find out the total soil microbial populations including fungi and bacteria and to correlate its presence with wilt incidence. After rainy season when the conditions are favorable for incidence of wilt the data before onset of rain and after rainy season were correlated based on wilt incidences. *Bacillus*, *Pseudomonas, Fusarium oxysporum* f. sp. *psidii*, *F. solani*, *Trichoderma* and *Aspergillus niger,* were the major population recorded from all locations. Twenty one *F. oxysporum* f. sp. *psidii* isolates were identified and purified from infected guava root and soil samples of different agro-ecological regions and were maintained on potato dextrose agar medium. Based on pigmentation, the isolates were grouped into separate groups, ranging from pink, brown, reddish brown and creamy red.

Genetic diversity studies

The PCR based on the enterobacterial repetitive intergenic consensus sequence, the repetitive extragenic palindromic sequence and the BOX element were used to evaluate the diversity in *F. oxysporum* f. sp. *psidii* isolates.

Sequencing of the partial region of ITS + 5.8s rDNA using ITS1 primer was under taken and submitted to GenBank and the pathogenic isolates were found to be highly variable within the ITS regions. The NUPACK tool was used for designing of secondary structure of ITS sequences based on minimal free energy state.

b. DIAGNOSTICS

Direct nested PCR detection assay for diagnosis of *F. oxysporum* f. sp. *psidii*

A colony PCR tool has been developed to amplify the internal transcribed spacer (ITS) region of fungal rDNA of *F. oxysporum* f. sp. *psidii* isolates. Three sets of primers were used which resulted in amplicon size of 570, 230 and 280 bp, respectively.

Amplification of ITS region

The ITS1-5.8S-ITS2 region of the *Fusarium* spp. was amplified using the ITS1 and ITS4 primers which resulted in approximately 570 bp fragment in all the isolates. This indicates that there was no size variation in the ITS + 5.8S rDNA region among *Fusarium* isolates. To detect *Fusarium* spp. and *F. oxysporum* f. sp. *psidii,* primer pairs, ITS1F/ ITS1R and 172F/447R, were used which amplified expected size-diagnostic bands, 230 bp for *Fusarium* spp. and 380 bp for *F. oxysporum* f. sp. *psidii*. Isolate- 206 (*Fusarium* sp.) from Gopalganj (Bihar) was amplified with primer ITS1F/ITS1R but no amplification was obtained with primer 172F/ 447R.

Nested PCR amplification

The nested PCR included two rounds of amplification using the universal primers ITS1/ITS4. (*Fusarium* spp. primer ITS1F/ITS1R for the first round and *F. oxysporum* f. sp. *psidii* specific primers 172F/447R for the second round). The specificity and sensitivity of the primer sets ITS1F/ ITSR and 172F/447R was shown by amplification of 230 and 280 bp products from second round of PCR amplification in all the isolates of *Fusarium* spp. Also in the nested PCR, no amplification was obtained in Fusarium-206 with primer 172F/447R.

To determine the sensitivity and specificity of the direct colony PCR, serial dilutions of reference fungal isolate from the corresponding species were tested. Fig. 34 and Fig. 35 shows the results of agarose gel electrophoresis of representative PCR products of the serial dilution of the fungal strains with primers ITS1F/ITS1R and 172F/447R; and the sensitivity of each set of the tests ranged up to 10^6 and 10^7 dilution, respectively, which indicates the potential to amplify the DNA directly from cultures.

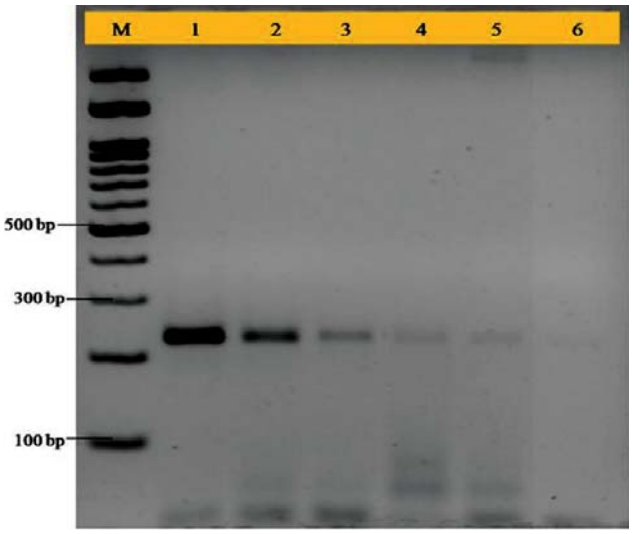


Fig. 34. Sensitivity of PCR using primers ITS1F/ITSIR with *F. oxysporum* f. sp. *psidii* suspension. Lane M, 100-bp DNA ladder marker; Lanes 1–6, dilution were 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6, respectively

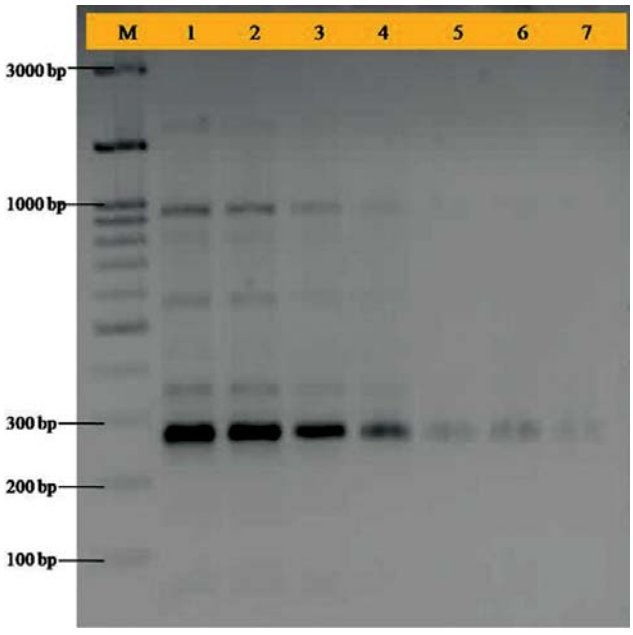


Fig. 35. Sensitivity of PCR using primers 172F/447R with *F. oxysporum* f. sp. *psidii* suspension. Lane M, 100-bp DNA ladder marker; Lanes 1–7, dilution were 10^1, 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7, respectively

Amplificaton of translation elongation factor 1α gene and PCR RFLP of *F. oxysporum* f. sp. *psidii*

To investigate the genetic relationship within *F. oxysporum* f. sp. *psidii* isolates, translation elongation factor 1α gene (*TEF*-1α) and internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) were amplified with primer EF1F-EF1R and ITS1-ITS4, respectively (Fig. 36 and 37). After the amplified products were digested with restriction enzymes, restriction fragment length polymorphism (RFLP) patterns were analyzed (Fig. 38 and 39). Both *Msp*I and *Hae*III gave prominent and discriminating banding patterns in RFLP analysis. Enzymatic digestions of both regions generated specific RFLP markers for each isolates and subsequently revealed some intra-specific variations in this region of rDNA. Electrophoresis result showed similar type of restriction pattern by both the primers. Based on the common RFLP data analysis, the 21 isolates were divided into four RFLP types. The results indicate that RFLPs generated by restriction enzyme digestion with *Msp*I of PCR amplified rDNA can be used for discrimination of *F. oxysporum* f. sp. *psidii* isolates.

The dendrogram generated (Fig. 40) using the combined data of *TEF*-1α gene and ITS RFLP score generated four main clusters. *Fop* -10, 18, 37, 135, 134, 203, 201, 48, 148, 23, 19, 128, 127 and 123 were found in a major clade I, having similar type (100%) of restriction sites. They produced two major fragments of approximately 120 and 520 bp of TEF-1α gene while 220 and 350 bp of ITS region. Isolate F-147, 44 and *Fop*-137 grouped in clade II. Isolates *Fop*-14, *Fop*-24 and 146 were in clade III in which *Fop*-14 and *Fop*-24 had 56% similarity. *Fop*-146 had 26% similarity to Fop-14 and *Fop*-24. *Fop*-125 was found different from all the isolates having different restriction sites and showed only 14.66% similarity in clade IV. Based on the common RFLP data analysis, the 21 isolates were divided into four RFLP types.

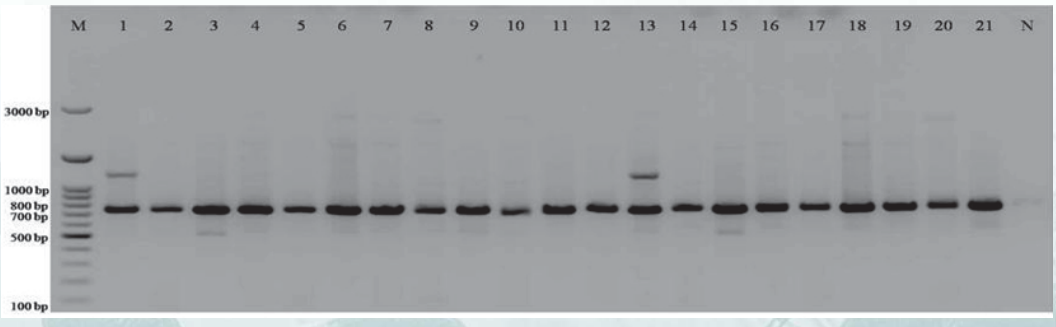


Fig. 36. PCR products using primers EF1F and EF1R specific for amplifying 750 bp fragment. Lane M: 100 bp DNA ladder, Lane 1-21: *F. oxysporum* f. sp. *psidii* isolates, Lane N: negative control

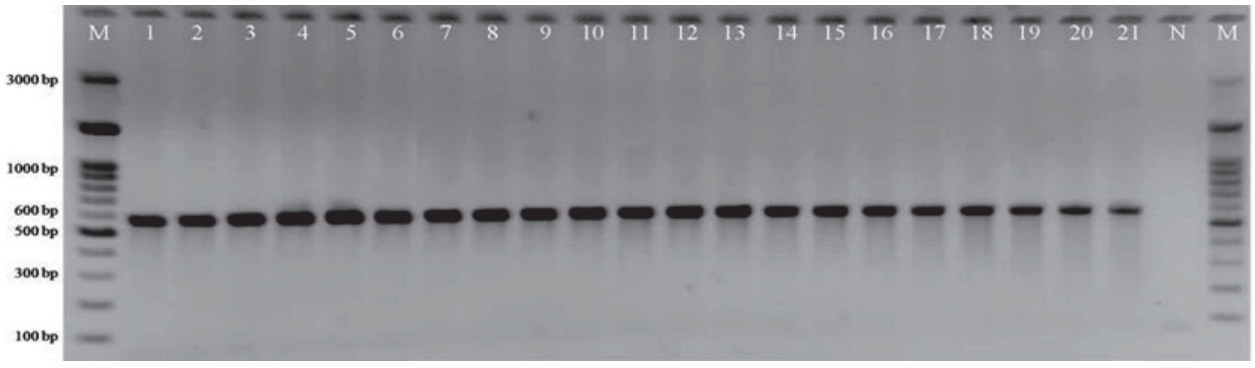


Fig. 37. PCR products using primers ITS1 and ITS4 specific for amplifying 570 bp fragment. Lane M: 100 bp DNA ladder, Lane 1-21: *F.oxysporum* f. sp. *psidii* isolates, Lane N: negative control

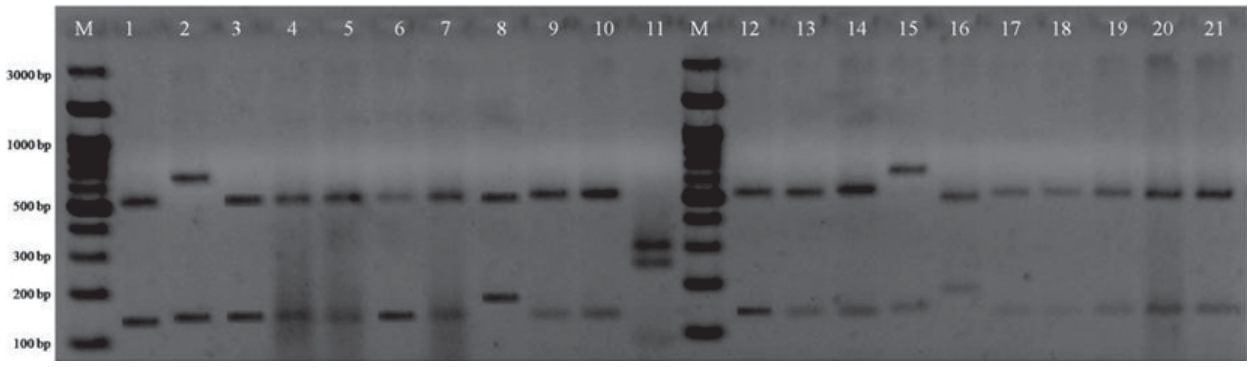


Fig. 38. Agarose gels showing restriction patterns of PCR-amplified TEF-1α gene digested with *Msp*I. Lane M: 100 bp DNA ladder and Lane 1-21: *Fop* isolates

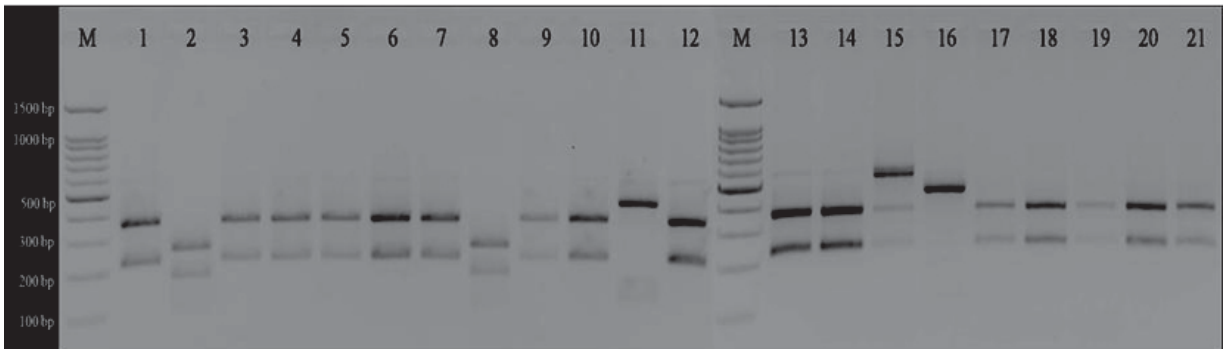


Fig. 39. Agarose gels showing restriction patterns of PCR-amplified ITS+5.8S rDNA digested with *Msp*I. Lane M: 100 bp DNA ladder and Lane 1-21: *Fop* isolates

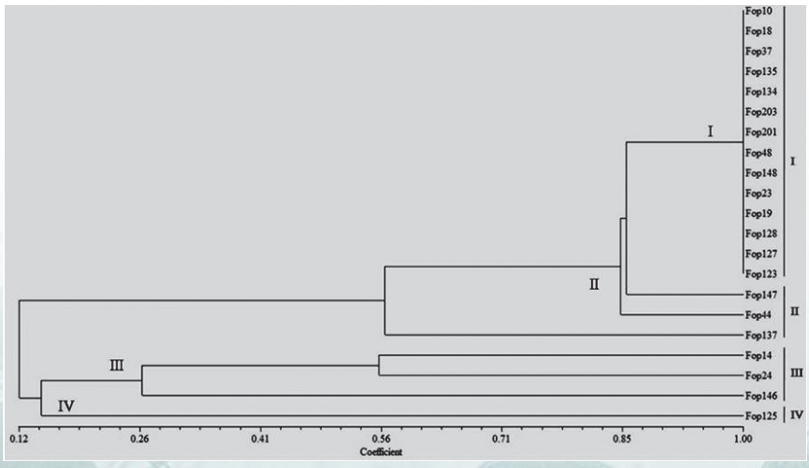


Fig. 40. Combined UPGMA dendogram showing relationships among the 21 isolates of *F. oxysporum* f. sp. *psidii* based on restriction site data

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a. *FUSARIUM* DIVERSITY

A total of seven and five *Phytophthora* isolates were collected from the safflower and castor crops, respectively.

b. DIAGNOSTICS

To know the variant sequence which encodes wilt in safflower caused by *F. oxysporum* f. sp. *carthami,* a total of 20 different gene sequences for *F. oxysporum* were obtained from NCBI and multiple sequence alignment was undertaken. The qPCR protocol is under standardization to amplify the *F. oxysporum* f. sp. *carthami* in the early and mixed infections by using Syber Green and custom made TaqMan assays, respectively. Based on the findings, custom made TaqMan assay is well suited for amplification of specific region of wilt caused by *F. oxysporum* f. sp. *carthami* in safflower.

c. GENOMICS

Identification of species specific molecular markers for targeted resistant genes in *Carthamus* wild species

A total of 43, 17, 6, 11, 9 and 7 EST-SSR markers specific to wild species *viz., C. oxyacantha, C. palaestinus, C. lanatus, C. creticus, C. glaucus* and *C. turkistanicus,* respectively, have been identified (Fig. 41) and the work is in progress for identifying the EST-SSR markers flanked to wilt resistance using Bulk Segregant Analysis (BSA). In addition, recently synthesized 141 genomic SSRs are being screened for identifying the polymorphic markers.

Inheritance study on wilt resistance

Inheritance of wilt resistance in crosses between susceptible cultivated species (Nira) and resistant wild species *viz.*, *C. lanatus* (2*n*=44), *C. creticus* ((2*n*=44), *C. glaucus* (2*n*=20), and *C. turkistanicus* (2*n*=64) could not be studied during 2011-12 owing to very low plant stand because of water logging condition in wilt sick plot after heavy

Fig. 41. Confirmation of resistant source using BSA in F_2 population of *C. tinctorius* (Nira) x *C. oxyacantha* (IP-16) with ESSR-33. Lanes: 1. Nira (*C. tinctorious*); 2. IP-16 (*C. oxyacantha*); 3. Resistant bulk; 4-7. Resistant individuals; 8. Susceptible bulk; 9-12. Susceptible individuals

rainfall and low incidence of wilt in wilt sick plot. The crosses between Nira and *C. lanatus* (2*n*=44), *C. creticus* ((2*n*=44), *C. glaucus* (2*n*=20), and *C. turkistanicus* (2*n*=64) were made again during 2011. These F_1's are being phenotyped for reaction against *F. oxysporum* f.sp. *carthami*. In addition, the F_2 generations of the crosses between the susceptible cultivated species (Nira), resistant wild species (*C. oxyacntha* and *C. palaeastinus*) and susceptible *C. tinctorius* (Nira) x resistant *C. tinctorius* (96-508-2-90) are being screened against wilt in sick plot. So far wilt data was collected from one month after sowing with 15 day intervals. Resistance reaction was observed in F_2 generations of these crosses.

d. HOST RESISTANCE

Systemic acquired resistance for *Fusarium* wilt by bioagents

Increased accumulation and activity of defense related enzymes *viz.,* peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) in three potential *Trichoderma* spp. *viz., T. harzianum* (Th4d), *T. viride* (Tv5) and *T. asperellum* (T7316) pre-inoculated safflower plants challenged with *F. oxysporum* f. sp *carthami* was observed.

Peroxidase activity at 30 days interval began to increase 24 h after challenge inoculation and the activity reached maximum at 72 h. PAL activity reached maximum at 48 h of inoculation. Similarly PPO activity increased significantly within 24 h after treatment and reached maximum level at 72 h of

inoculation there after reduction in the activities of polyphenol oxidizing enzymes was recorded when plants treated with both *F. oxysporum* f. sp *carthami* and antagonist compared to control alone (Fig. 42).

e. DISEASE MANAGEMENT

Management of *Phytophthora* damping off and seedling blight of safflower by seed treatment with chemical and biological agents

The trial on management of this disease was conducted at DOR, Hyderabad and Phaltan, Maharashtra. *Trichoderma harzianum* Th4d SC @ 1 ml gave the significantly highest seed yield. Significantly lowest wilt incidence was obtained with treatments of cymoxanil + mancozeb, metalaxyl + mancozeb, *T. harzianum* Th4d SC and *P. fluorescens* Pf2 (Table 22).

Table 22. Management of *Phytophthora* damping off of safflower by seed treatment with chemical and biological agents

Treatment	Disease incidence (%)
Carbendazim + Mancozeb (Saff) @ 2 g/kg	20.6
Captan @ 2 g/kg	11.8
Metalaxyl + Mancozeb (Ridomil MZ) @ 2 g/kg	15.3
T. harzianum Th4d 1 ml/kg	15.6
T. harzianum Th4d 2 ml/kg	16.3
P. fluorescens Pf2 @ 5 g/kg	26.4
P. fluorescens Pf2 @10 g/kg	17.6
Pathogen check	51.6
CD (P=0.05)	3.5
CV (%)	8.8

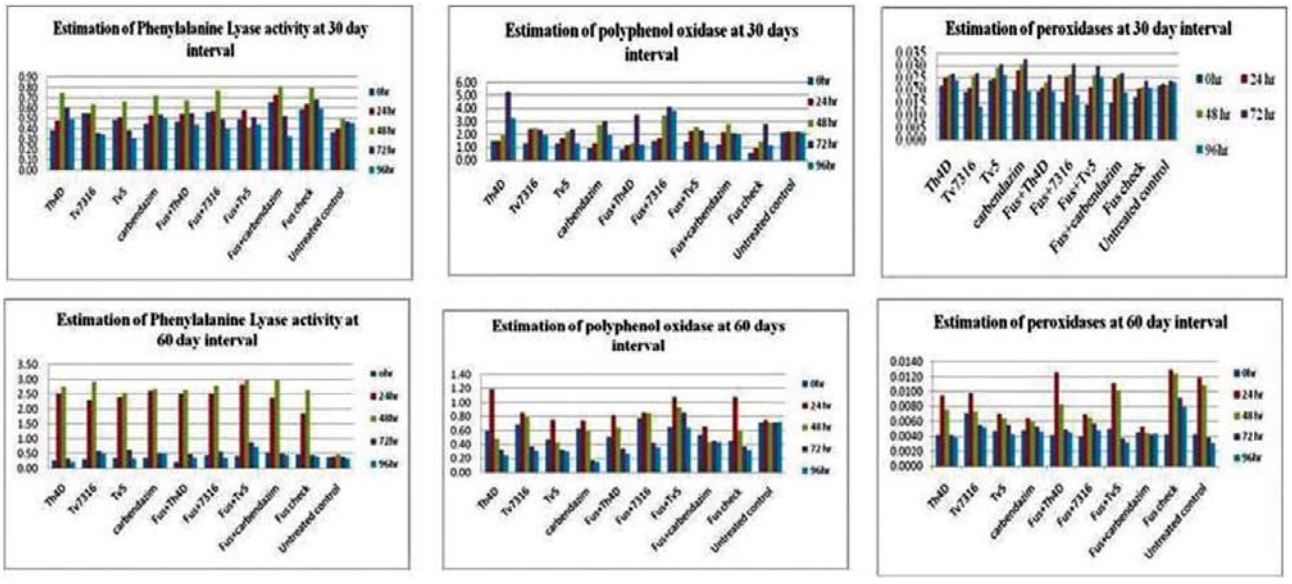

Fig. 42. Activity of different defense related enzymes

Management of seed/soil borne diseases of safflower by seed treatment with chemical and biological agents

The incidence of *Fusarium* wilt and *Macrophomina* root rot were significantly low in different chemical and biological agents seed treatments (Fig. 43, Table 23). Among the different seed treatments, Captan @ 0.2% was found to be the most effective recording significantly least incidence of *Fusarium* wilt (10.3%) and *Macrophomina* root rot (8.2%). The untreated recorded the highest incidence of *Fusarium* wilt (33.3%) and *Macrophomina* root rot (23.9%). Significantly higher seed yield (2150 kg/ha) was recorded in *T. harzianum* Th4d treatment followed by Captan (1822 kg/ha) treatment.

Fig. 43. Management of wilt and root rot disease complex of safflower by seed treatment with chemical and biological agents

Table 23. Incidence of seed/soil borne diseases of safflower as influenced by seed treatment with chemical and biological agents

Treatment	*Fusarium* wilt (%)	*Macrophomina* root rot (%)	Seed yield (kg/ha)
Carbendazim + Mancozeb (SAFF) @ 0.2%	16.3	7.4	1783
Carbendazim @ 0.2%	20.2	6.2	1416
Captan @ 0.2%	10.3	8.2	1822
Cymoxanil 8% + Mancozeb 64% @ 0.2%	18.8	10.3	1611
Metalaxyl + Mancozeb (Ridomil MZ) @ 0.2%	12.9	11.9	1661
T. harzianum Th4d SC @ 2 ml / kg	18.3	11.0	2150
P. fluorescens Pf2 WP @ 5 g/kg	24.3	13.4	1605
Untreated check	33.3	20.6	911
CD (P=0.05)	1.7	1.2	260.1
CV (%)	7.1	9.1	10.1

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IGS Analysis

Intergenic spacer region (IGS) was exploited to determine the genetic diversity of 22 isolates of *F. oxysporum* f. sp. *ciceris* (*Foc*) originating from 13 states of India representing different races of the pathogen. Partial IGS region was amplified by using a set of universal primers, namely, CNS1 and U46.67 which produced 1000 bp fragment in all the isolates of the pathogen and sequenced. The phylogenetic tree constructed using bootstrap neighborhood-joining analysis, grouped the isolates into two major clusters. Except one isolate from Andhra Pradesh (*Foc* 118), all were grouped into single cluster representing different races as well as places of origin indicating high level of similarity among the isolates in respect of partial IGS gene sequences. The groups did not correspond to state of origin as well as races of the pathogen (Fig. 44).

IGS-RFLP analysis

The IGS region of 22 representative isolates was amplified with universal primers CNS1 and CNL12 and digested with 10 restriction enzymes, namely, *EcoRI*, *Hind III*, *Ava I*, *Ms 1I*, *Bpu 10 I*, *Bsm AI*, *Alu I*, *Hph I*, *Mbo I*, *BfuCI* and *Msp I*. A restriction pattern of *Foc* isolates with the enzymes *Alu I* is given in Fig. 45.

b. DIAGNOSTICS

α-tubulin gene (B164 F1 and R1) and IGS region based (ISR 52 F1 and R1) primers were designed from the sequences of respective genes. The PCR yielded the fragment size of 300 bp and 325 bp, respectively, for *Foc* only and did not produce any

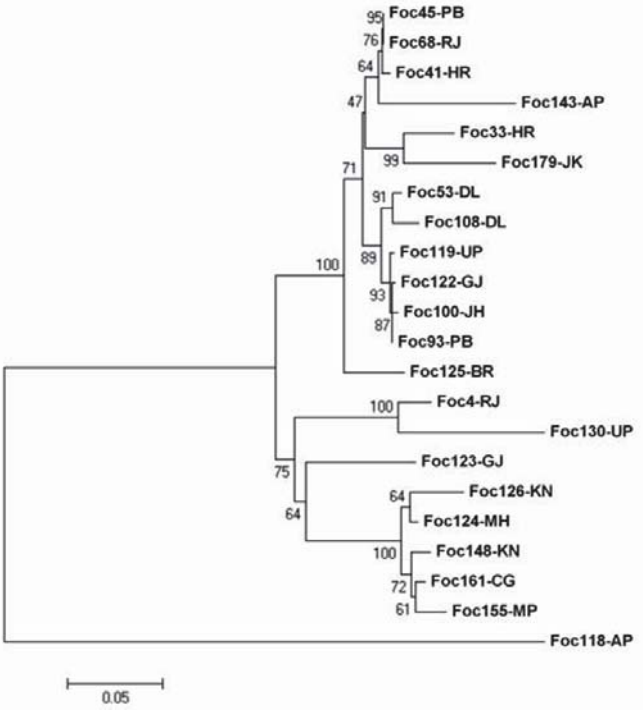

Fig. 44. Neighborhood joining tree showing the phylogenetic relationships among isolates of *F. oxysporum* based on their IGS sequences

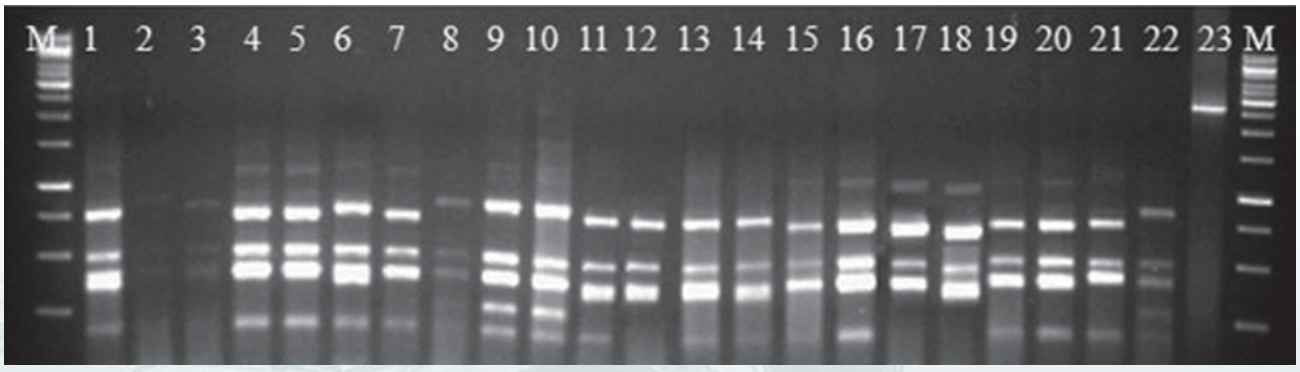

Fig. 45. IGS- RFLP analysis of *Foc* isolates with the enzymes *Alu I*. M= marker 1kb; lanes 1-2 (Andhra Pradesh), 3 (Chattisgarh), 4-5 (Delhi), 6-7 (Gujarat), 8-9 (Haryana), 10 (Jharkhand), 11-12 (Karnataka), 13 (Madhya Pradesh), 14 (Maharashtra), 15-16 (Punjab), 17-18 (Rajasthan), 19-20 (Uttar Pradesh), 21 (Bihar), 22 (Jammu and Kashmir) and lane 23 (undigested PCR product) isolates of *Foc*

amplification with other representative test plant pathogenic fungi. The markers were also able to detect the pathogen in infected chickpea plants. Primer ISR-52 F1R1 had the ability to detect the minimum concentration of 50 pg and 1 ng in *Foc* and infected plant samples, respectively. The sensitivity or minimum detection limit of the assay was estimated to quantify and detect the lowest amount of target DNA in real time assay and was found that the minimum detection limit of B 164 F1 and R1 primer was 0.01 pg for *Foc* and 0.05 pg for infected plant samples. Similarly, the primer ISR-52 F1R1 was able to detect the minimum concentration of 0.1 pg and 1 pg in *Foc* and infected plant samples, respectively (Fig. 46 and 47).

c. HOST PATHOGEN INTERACTION

Highly susceptible chickpea variety JG 62 and resistant variety GPF 2 were selected for defense gene analysis using real time PCR. Chickpea seedlings at the stage of 2-3 leaves were drenched with salicylic acid, jasmonic acid and *Trichoderma* prior to *Foc* infection (10^6 spores/ml) separately and in combinations. Un-inoculated plant samples were used as a control. The plant samples obtained from eight different treatment (T1 - *Foc*, T2 - uninoculated (without *Foc*), T3 - salicylic acid + *Foc*, T4 - salicylic acid, T5 - jasmonic acid + *Foc*, T6 - jasmonic acid , T7 - *T. harzianum* + *Foc*, T8 - *T. harzianum*) combinations for each chickpea variety, were used for RNA isolation and cDNA preparation at 1-4 days after inoculation (dai). The assessment of gene expression ratio at 1 dai and 4 dai between treated and untreated susceptible (JG 62) and resistant (GPF 2) cultivars of chickpea performed by using conventional (Fig. 48) and real time PCR showed that the relative expression of chalcone synthase was up-regulated in JG 62 at 1 dai in response to *Foc* (T1), salicylic acid (T4),

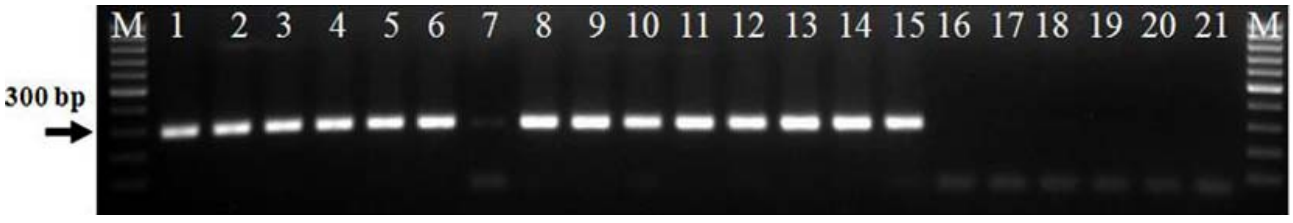

Fig. 46. PCR amplication products of genomic DNA of *Foc* isolates using *Foc* specific markers B 164 F1R1. Lane 1-14 - *Foc* isolates, lane 15 - *Foc* inoculated plant sample, lane - 16 un-inoculated plant sample, lane 17 - *F. solani,* lane 18 – *F. udum*, lane 19 - *Rhizoctonia solani*, lane 20 - *Sclerotinia sclerotiorum,* lane 21 – NTC and M – 100 bp ladder on both sides

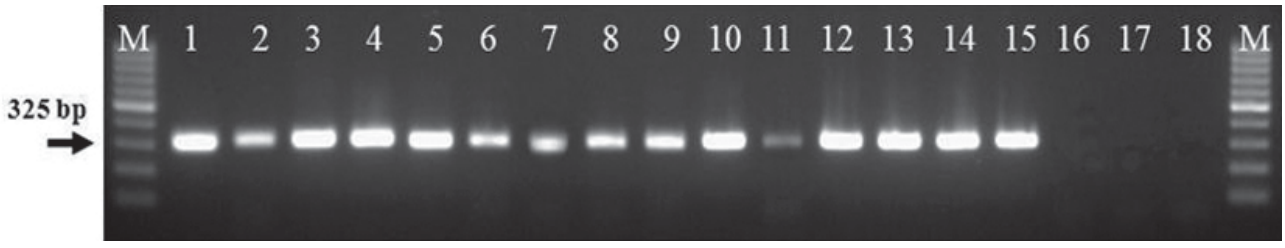

Fig. 47. PCR amplication products of genomic DNA of *Foc* isolates using *Foc* specific markers ISR 52 F1R1. Lane 1-14 - *Foc* isolates, lane 15 – *Foc* inoculated plant sample, lane 16 - un-inoculated plant sample, lane 17 - *Rhizoctonia solani*, lane 18 - *Sclerotinia sclerotiorum,* and M – 100bp ladder on both sides

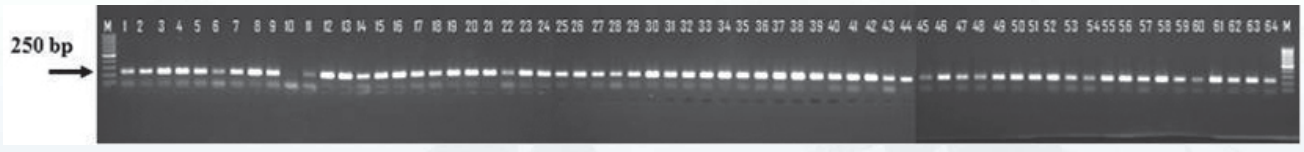

Fig. 48. Expression profile generated by chalchone synthase primer. Lanes 1-4 (V1-*Foc*, 1-4 dai), 5-8 (V1- uninoculated, 1-4 dai)), 9-12 (V1-Salicylic acid + *Foc*, 1-4 dai), 13-16 (V1-Salicylic acid, 1-4 dai), 17-20 (V1-Jasmonic acid+*Foc*, 1-4 dai), 21-24 (V1-Jasmonic acid, 1-4 dai), 25-28 (V1- *Trichoderma*+*Foc*, 1-4 dai), 29-32 (V1- *Trichoderma*, 1-4 dai), 33-36 (V2-*Foc*, 1-4 dai), 37-40 (V2- uninoculated, 1-4 dai)), 41-44 (V2- Salicylic acid+*Foc*, 1-4 dai), 45-48 (V2- Salicylic acid, 1-4 dai), 49-52 (V2- Jasmonic acid+*Foc*, 1-4 dai), 53-56 (V2- Jasmonic acid, 1-4 dai), 57-60 (V2- *Trichoderma*+*Foc*, 1-4 dai), 61-64 (V2- *Trichoderma*, 1-4 dai) and M- 100 bp ladder. [V1-JG62, V2-GPF2]

jasmonic acid (T6) and *T. harzianum* + *Foc* (T7) as compared to the control, whereas in resistant cultivar GPF 2, it was down-regulated in all treatments including *Foc* inoculated plants. Plant (JG 62) treated with salicylic acid (T4) showed the highest up regulation, while treatment of *T. harzianum* (T8) showed the highest down regulation in both the varieties at 1 dai. The transcripts level of chalcone synthase of plant in response to *Foc* (T1), salicylic acid + *Foc* (T3) and jasmonic acid (T6) was increased drastically in JG 62 at 4 dai, however, in GPF 2, the expression level was up-regulated in response to *Foc* inoculated (T1), salicylic acid + *Foc* (T3), salicylic acid (T4) and jasmonic acid + *Foc* (T5). The treatment of jasmonic acid (T6) showed the highest up-regulation in JG 62 and *T. harzianum* (T8) showed the highest down regulation in both the varieties at 4 dai whereas, jasmonic acid + *Foc* (T5) showed the highest up-regulation in GPF 2 at 4 dai (Fig. 49).

d. DISEASE MANAGEMENT

Integrated management

The field experiments were conducted at two locations, one in a sick field (with *Foc*) and another in a non-sick field at the IARI Research Farm, New Delhi. Chickpea wilt susceptible cultivar Pusa 362 was sown at 30 cm x 10 cm spacing with a plot size of 6 m^2 in three replications. Seeds were treated with bioagents and fungicide separately and in combination as per treatment before sowing. Pusa 5SD and *P. fluorescens* were used at 4 g kg^{-1} of seed. The fungicide carboxin + thiram (Vitavax power™) and the mixture of carbendazim (Bavistin™) + tetramethyl thiuram disulphide (Thiram™) (1:1 ratio) were used at 2 g kg^{-1} of seed while carboxin + thiram (Vitavax power) was used at 1g kg^{-1} of seed when combined with bioagent. Seed germination was counted 15 days after sowing. Wilt incidence was recorded at 20 days intervals up to the maturity of the crop and total wilted plants per plot were presented. Grain yield was measured after harvest of the crop.

All the seed treatments evaluated significantly ($p<0.05$) enhanced the seed germination and the grain yield of chickpea and reduced the wilt incidence as against those of the control. A combination of Pusa 5SD (*T. harzianum*) + carboxin + TMTD (Vitavax power™) + *P. fluorescens* (*Pf*-80) provided significantly higher seed germination and grain yield compared to those of other treatments. The lowest wilt incidence was also recorded in this treatment and the wilt incidence recorded in this treatment was not statistically different from that of carboxin + Thiram (Table 24 and 25).

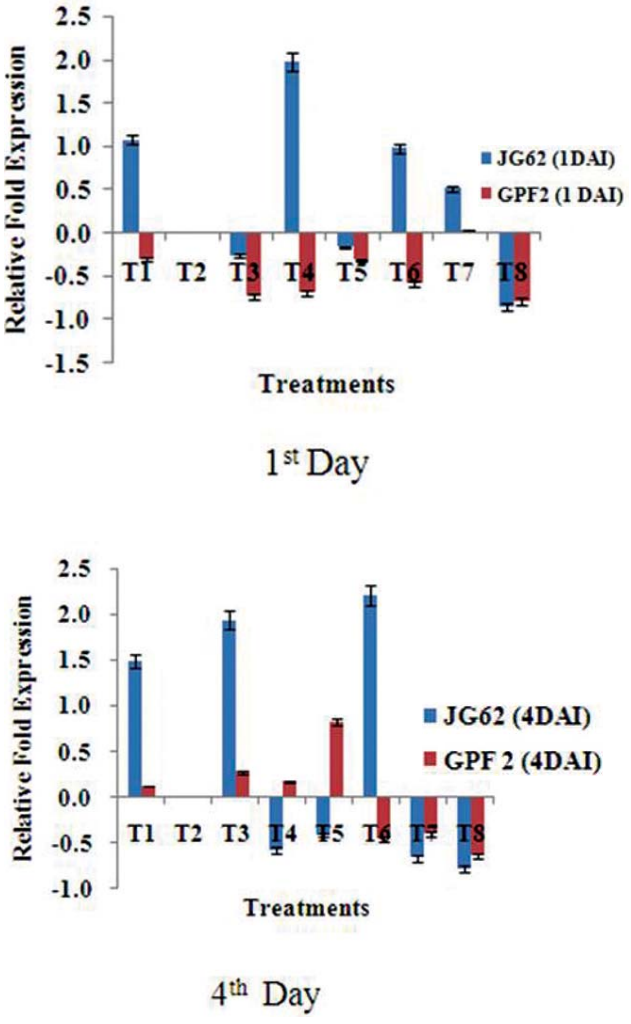

Fig. 49. Expression profiling of chalchone synthase gene at various days post inoculation (a-1 day and b- 4 days) in 8 treatments [T1 - *Foc*, T2 - un-inoculated plant sample, T3- Salicylic acid + *Foc*, T4 - Salicylic acid, T5 - Jasmonic acid + *Foc*, T6 - Jasmonic acid , T7 - *T. harzianum* + *Foc*, T8 - *T. harzianum*]

Evaluation of *PhytoFuRa* isolates of *Trichoderma* species against *F. oxysporum* f. sp. *ciceris* in pot experiment

The pot experiment in CRD was conducted during 2011-12 and 2012-2013 to evaluate the performance of *PhytoFuRa* isolates of *Trichoderma*

Table 24. Effect of seed treatments on seed germination, wilt incidence and grain yield of chickpea cultivar Pusa 362 under field condition

Treatment	Seed germination (%)	Wilt incidence (%)	Grain yield (kg ha^{-1})
Pusa 5SD (*T. harzianum*)	76.1 (60.7)^{def}	6.3 (14.5)^{ef}	1191.7^{d}
P. fluorescens 80 (*Pf* 80)	72.8 (58.6)^{fg}	6.5 (14.8)^{f}	1033.3^{f}
Carboxin + TMTD (Vitavax power^{TM})	80.4 (63.8)^{bc}	4.5 (12.2)^{bc}	1275.0^{bc}
Pusa 5SD + *Pf* 80	78.9 (62.7)^{cd}	5.9 (14.1)^{def}	1241.7^{cd}
Pusa 5SD + Vitavax power^{TM} + *Pf* 80	83.1 (65.7)^{ab}	3.2 (10.3)^{a}	1541.7^{a}
Carbendazim (Bavistin^{TM}) + TMTD (Thiram^{TM})	74.7 (59.8)^{ef}	4.6 (12.4)^{c}	1095.8^{ef}
Control (untreated seeds)	67.4 (55.2)^{g}	9.7 (18.1)^{g}	937.5^{g}

The figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

Table 25. Effect of seed treatments on seed germination, wilt incidence and grain yield of chickpea cultivar Pusa 362 under wilt sick field condition

Treatment	Seed germination (%)	Wilt incidence (%)	Grain yield (kg ha^{-1})
Pusa 5SD (*T. harzianum*)	77 (61.3)^{ef}	28.8 (32.5)^{d}	712.9^{de}
P. fluorescens 80 (*Pf* 80)	76.7 (61.1)^{fc}	33.1 (35.1)^{e}	648.2^{f}
Carboxin + TMTD (Vitavax power^{TM})	82.8 (65.5)^{bc}	18.5 (25.5)^{b}	861.1^{b}
Pusa 5SD + *Pf* 80	79.2 (62.9)^{def}	23.9 (29.3)^{c}	740.7^{cd}
Pusa 5SD + Vitavax power^{TM} + *Pf* 80	86.7 (68.6)^{a}	16.3 (23.8)^{ab}	898.1^{a}
Carbendazim (Bavistin^{TM}) + TMTD (Thiram^{TM})	80.3 (63.7)^{cde}	36.9 (37.4)^{f}	694.4^{e}
Control (untreated seeds)	68.9 (56.1)^{g}	52.3 (46.3)^{g}	500^{g}

The figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

(*PhytoFuRa* 1-15) against wilt of chickpea. The results of 2012-13 indicated that the seed treatment effect was non significant on germination. The seeds treated with *PhytoFuRa* 10 provided the lowest wilt incidence followed by *PhytoFuRa*-1 (Table 26).

Table 26. Effect of seed treatment by different PhytoFuRa isolates of *Trichoderma* species on seed germination and wilt incidence of chickpea

Treatment	Seed germination (%)		Wilt incidence (%)	
	2011-12	2012-13*	2011-12	2012-13
PhytoFuRa-1	65.0 (53.8) ^{de}	100 (90.0)	61.9 (51.9) ^{bc}	63.6 (52.9)^{b}
PhytoFuRa-2	80.0 (63.4)^{ab}	100 (90.0)	75.0 (60.0) ^{def}	81.8 (64.7)^{de}
PhytoFuRa-3	75.0 (60.1)^{bcd}	100 (90.0)	67.0 (54.9) ^{bcd}	77.3 (61.6)^{d}
PhytoFuRa-4	65.0 (53.8) ^{de}	100 (90.0)	84.5 (66.8)^{f}	100.0 (90.0)^{h}
PhytoFuRa-5	75.0 (60.1) ^{bcd}	90.9 (77.4)	73.2 (58.8) ^{cde}	89.9 (71.5)^{fg}
PhytoFuRa-6	75.0 (60.1) ^{bcd}	90.9 (77.4)	80.4 (63.9)^{ef}	89.9 (71.5)^{fg}
PhytoFuRa-7	75.0 (60.1) ^{bcd}	100 (90.0)	73.2 (58.8) ^{cde}	86.4 (68.6)^{ef}
PhytoFuRa-8	75.0 (60.1) ^{bcd}	100 (90.0)	59.8 (50.7) ^{b}	77.3 (61.6)^{d}
PhytoFuRa-9	75.0 (60.1) ^{bcd}	100 (90.0)	67.0 (54.9) ^{bcd}	68.2 (55.7)^{bc}
PhytoFuRa-10	75.0 (60.1) ^{bcd}	100 (90.0)	40.2 (39.3) ^{a}	36.6 (37.2)^{a}
PhytoFuRa-11	70.0 (56.8) ^{bcde}	100 (90.0)	78.6 (62.7)^{ef}	68.2 (55.7)^{bc}
PhytoFuRa-12	80.0 (63.4)^{ab}	100 (90.0)	81.3 (64.6)^{ef}	100.0 (90.0)^{h}
PhytoFuRa-13	65.0 (53.8) ^{de}	100 (90.0)	84.5 (66.8)^{f}	90.9 (72.4)^{fg}
PhytoFuRa-14	90.0 (71.5)^{a}	90.9 (77.4)	77.8 (61.9)^{def}	89.9 (71.5)^{fg}
PhytoFuRa-15	75.0 (60.1) ^{bcd}	100 (90.0)	67.0 (54.9) ^{bcd}	81.8 (64.7)^{de}
Control (inoculated+ untreated seed)	60.0 (50.7) ^{e}	90.9 (77.4)	83.3 (65.9)^{ef}	100.0 (90.0)^{h}

* Seed germination for 2012-13 was non-significant. The figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

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Pigeonpea wilt (*F. udum*)

A partial distribution map of *F. udum* variants identified in different states of India was prepared. Uttar Pradesh has all the seven variants followed by Maharashtra, Karnataka (6 each); Madhya Pradesh, Bihar (5 each); Andhra Pradesh, Rajasthan (4 each), Haryana (3); Tamil Nadu, Jharkhand (2 each) and West Bengal (one).

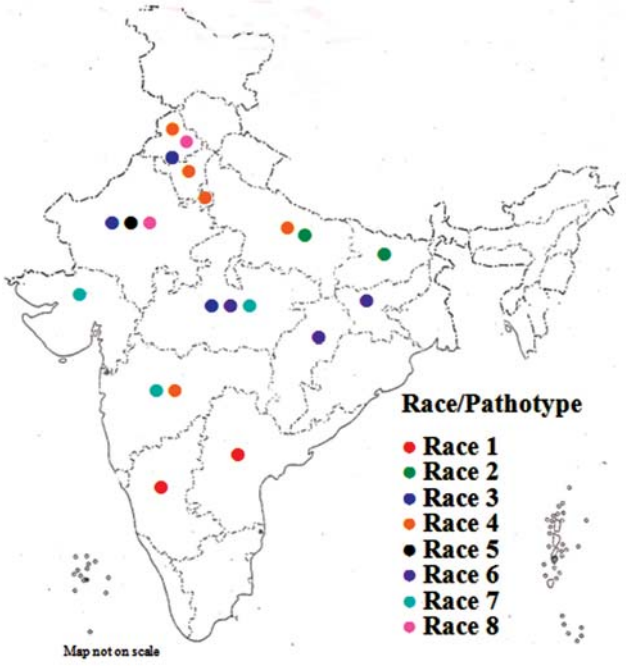

Fig. 50. Partial distribution map of *F. oxysporum* f.sp *ciceri* variants identified in different states of India

Chickpea wilt (*F. oxysporum* f.sp. *ciceri*)

A partial distribution map of *F. oxysporum* f.sp. *ciceri* races identified in different states of India was prepared (Fig. 50). Uttar Pradesh and Rajasthan have shown the presence of five races followed by Madhya Pradesh and Karnataka (four each); Haryana (three); Andhra Pradesh, Punjab and Gujarat (two each) and Chattisgarh, Delhi and Maharashtra (one each). Race 3 showed highest frequency with its presence in all the states followed by race 5 (five states), race 0, 1, 2 and 4 (four states each) and race 6 in 3 states.

Molecular characterization and diversity analysis of *F. oxysporum* f. sp. *ciceri* using SRAP and RGA marker combination

Fifty nine Indian isolates of *F. oxysporum* f. sp. *ciceri* from 12 chickpea growing states were screened with 80 SRAP and SRAP-RGA primer pairs and only 15 (four from SRAP-SRAP and 11 from SRAP-RGA primer combinations) showed good amplification. In UPGMA cluster analysis (Fig. 51) showed all isolates aligned into two main clusters at a similarity index value of 0.22. The first main cluster further divided into two sub-clusters at a similarity index value of 0.25. Sub-cluster I consists of 38 isolates which are further delineated into two sub groups, Sub group A contained 20 isolates of which most of them are from Uttar Pradesh and Rajasthan. Sub group B contained 18 isolates of which most of them are from Karnataka. Isolate K-68 from Andhra Pradesh held a separate position.

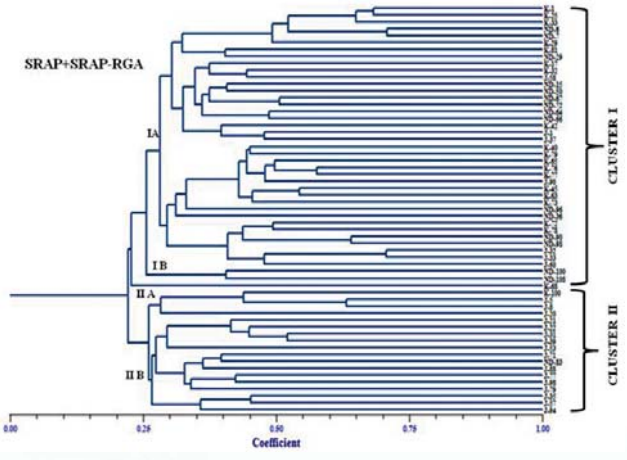

Fig. 51. UPGMA dendogram showing genetic relationship among 59 isolates of *Fusarium oxysporum* f. sp. *ciceri* based on SRAP and RGA analysis

Molecular characterization and diversity analysis of *F. udum* isolates using SRAP, ITS and EF-1 alpha marker

Forty five isolates of *F. udum* collected from 11 different geographical location of pigeonpea growing area in India were analyzed using sequence-related amplified polymorphism (SRAP)

markers, alpha transcription elongation factor (TEF-1 alpha) and nuclear ribosomal RNA (ITS) for comparative analysis and genetic diversity among 45 *F. udum* isolates. In UPGMA cluster analysis, three dendrograms derived from ITS, EF-1 alpha and combined analysis revealed three identical DNA profiles present in all five groups of isolates (F-3 and F-17; MSF-4 and MSF-12; I-3, I-8 and I-9; HF-1 and HF 23; NF-55 and NF-72).

The dendrogram constructed using unweighted pair-group method of arithmetic average cluster analysis (UPGMA) for 80 SRAP markers grouped the 45 isolates into two major clusters (cluster I and II) and one isolate clustered separately, indicating the existance of minimum three variants of pathogen.

b. DIAGNOSTICS

Development of diagnostic marker

Two SCAR markers were developed from the two successful RAPD markers. The amplification using race-2 and variant-1 specific SCAR markers showed amplification only in that particular race and variant, not in others (Fig. 52).

c. HOST RESISTANCE

Pigeonpea wilt (*F. udum*)

Four hundred and seventy nine pigeonpea genotypes comprising of wilt donors, differentials, promising lines, breeding lines, AICRP lines, germplasm, hybrids and ICAR-ICRISAT nursery lines were screened in wilt sick plot for identification of resistant genotypes. Twenty four donors, ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 89048, ICP 89049, ICP 9174, ICP 3012, BWR 377, AWR 74/15, BDN 1, Banda Palera, GPS 33, BSMR 736, BSMR 853, KPBR 80-2-1, PI 397430sel., PDE 92-2E, KPL 43, KPL 44, KPL 49, IPA 38 A, IPA 38B and IPA 40 were resistant in sick field. Out of 200 germplasm lines, only ICP 3993, PH 1059, PH 4713 and VKG 28171 were found resistant. Twelve pigeonpea lines from ICRISAT wilt nursery *viz*., ICPL nos. 20108, 20117, 20133, 20138, 20139, 20181, 99004, 99014, 99016, 99046, 99050 and ICP 8863 were found to be resistant in 2011-12. Similarly, out of 30 AICRP lines KPL43, GT101, TS3R, GRG818 and GRG 822 were resistant.

Seventeen pigeonpea lines were screened against variant 1, 2, 3, 4 and 5 under artificially inoculated sick tank conditions. Wilt development was low in variant 4 and 5 and moderate in variant 1, 2 and 3. Genotypes AWR 74/15, Banda Palera, MA 3, KPBR 80-2-1, ICP 8858, KPL 44, PI 397430 and IPF 9 were resistant to variant 1, 2 and 3.

Chickpea wilt (*F. oxysporum* f. sp. *ciceri*)

Total 40 chickpea lines found resistant in wilt sick field were screened against *F. oxysporum* f.sp. *ciceri* race 1, 2, 3, 4, 5 and 6 in the sick tanks. The

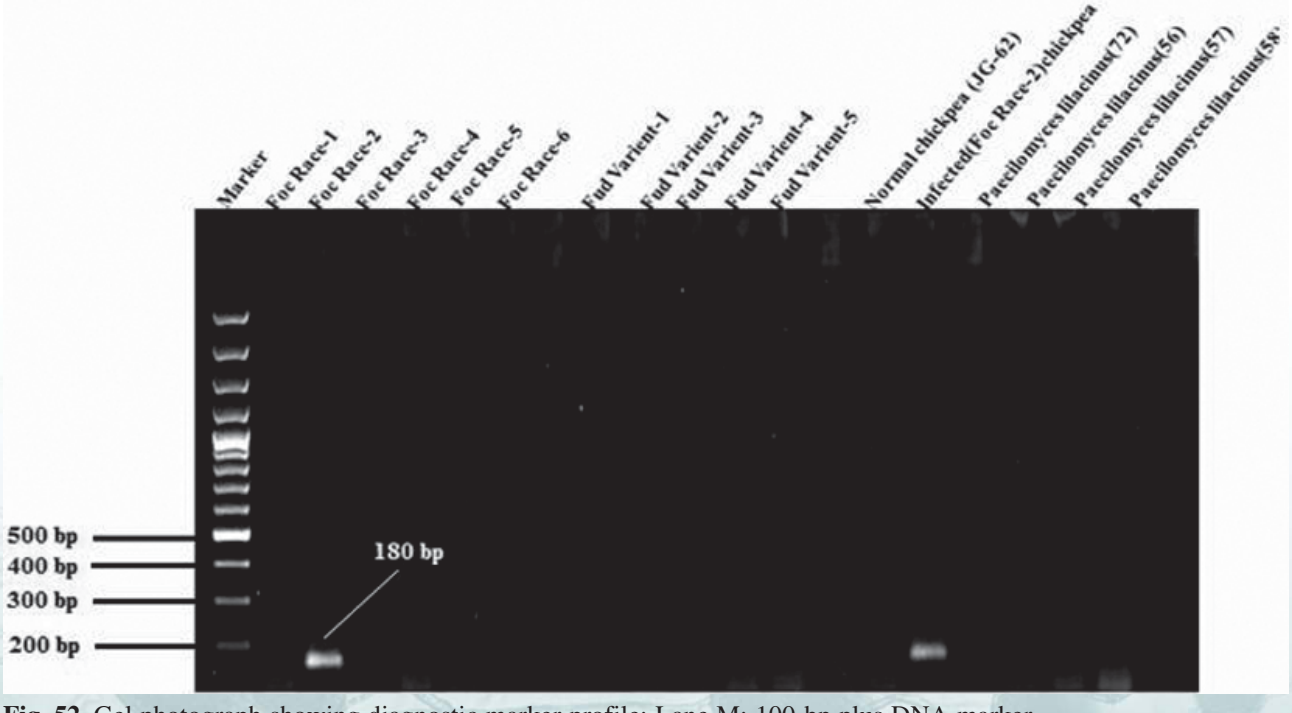

Fig. 52. Gel photograph showing diagnostic marker profile; Lane M: 100-bp plus DNA marker

lines *viz.,* IPC 2005-30, GNG 1861, IPC 2004-3, IPC 2005-41(A), IPC 2005-52, IPC 2005-41(B), IPC 2005-27, IPC 2004-52, IPC 2005-35, IPC 2005-37, IPC 2005-19, IPC 2005-43, IPC 2005-44, IPC 2005-62, IPC 2005-15, IPC 2004-8, IPC 2005-64 and CPS1 were resistant to moderately resistant to race 1, 2, 3, 4, 5 and 6 in 3rd year of screening.

d. DISEASE MANAGEMENT

Field experiment on pigeonpea wilt (*F. udum*)

Seed treatment with *Trichoderma* strain IPT 31 and carbendazim improved plant stand (14.3-14.8/m^2 against 11.8 in untreated). Significant reduction of wilt disease was noticed with *Trichoderma* strains IPT 31 and IPT 11 (9.5-10.8% against 23.7% in control). Wilt incidence in seed treatment alone gave 17.7% wilting while seed treatment + surface spray showed 13.5% overall incidence. Effect of soil surface spray was good only in *Trichoderma* strains IPT 31, and IPT 11. All treatments except *Trichoderma* strain from Bangaluru resulted 198-255 kg/ha higher yield over control. Seed treatment + spray again recorded 407 kg/ha higher yield over seed treatment alone.

Chickpea wilt (*F. oxysporum* f.sp. *ciceri*)

Trichoderma strains with code nos. 5, 9, 10, 13 and 14 were highly efficient in wilt incidence reduction (< 10%) followed by 3, 4, 11 and 12 (>10-15%). Least effective strains were 1, 2, 6, 7, 8, 15 and salicylic acid (>15-21%) as against 26.3% in control in a pot experiment with national collection of 15 *Trichoderma strains* and 10 ppm salicylic acid.

Antagonistic potential of non-pathogenic *Fusarium*

Four strains of non-pathogenic *Fusarium* (received from National Coordinator, Phytofura) were evaluated against *F. udum* and *F. oxysporum* f. sp. *ciceri* (*Foc*) for their inhibitory effects in dual culture. The non-pathogenic strain Fu-4 inhibited *F. udum* by 85.7% while Fu-7 inhibited *F. udum* and Foc by 47.6 and 31.4%, respectively. Other two strains Fu-24 and Fu-25 were less inhibitory to these pathogens revealing that Fu-4 and Fu-7 showed good potential, which could be explored further for the management of these two pathogens.

Suitability of crop wastes and organic substrates for multiplication and survival of *T. harzianum*

The multiplication potential of *T. harzianum* strain IPT 31 as the length of the column in the test tubes colonized by *Trichoderma* was recorded at 72 h. Sugarcane chaff supported best growth (11.0 cm.) followed by chickpea and pigeonpea straw, sorghum grain and neem seed (7.0 cm.) and slow growth (3.6-5.9 cm.) on farm yard manure, cow dung and goat dung. Observations on the survival of *Trichoderma* strain on these substrates (cfu/g dry substrate) after six months of inoculation showed 3.3-8.3 x 10^{10} cfu/g powder. Though highest population was achieved in sorghum grain, sugarcane, chickpea and pigeonpea straw, FYM and neem seed showed on par population among the crop residues and other organic substrates followed by goat dung. Cow dung showed poor population of *Trichoderma.*

INDIAN INSTITUTE OF VEGETABLE RESEARCH, VARANASI

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a. *FUSARIUM* DIVERSITY

Fusarium isolates, 19 of *F. solani* and 22 of *F. oxysporum,* were amplified and sequenced with chitin synthase gene primers (CHS79-F 5-TGG GGC AAG GAT GCI TGG AAG AAG-3 and CHS354-R 5-TGG AAG AAC CAT CTG TGA GAC TTG-3). Total 17 isolates of *F. solani* and seven isolates *F. oxysporum* showed positive response to the primer and yielded 300 bp fragment of CHS gene.

b. DIAGNOSTICS

ITS sequence information of 45 isolates of *Fusarium* spp. (20 isolates of *F. o.* f.sp. *lycopersici*, five of *F. oxysporum* and 20 of *F. solani*) were submitted to NCBI GeneBank.

Total 10 ISSR primers were tested on 14 isolates of *F. solani* and 18 isolates of *F. oxsporum*, out of which ISSR-4 (5- GCAACACACACACACAC-3) showed significant polymorphism in both the species. The polymorphic band of size 750 bp in *F. solani* and 1kb in *F. oxysporum* was sequenced to use it as a SCAR marker.

c. HOST RESISTANCE

Screening of tomato germplasm lines for resistance against wilt disease under field conditions

The germplasm lines of tomato were tested for resistance against wilt disease. Among them 2102-10-1, EC-620424, WIR- 4360, EC-620428, 2103-1-2, H-88-78-2 and C-11-1-4 were identified as resistant lines under field conditions (Fig. 53, Table 27).

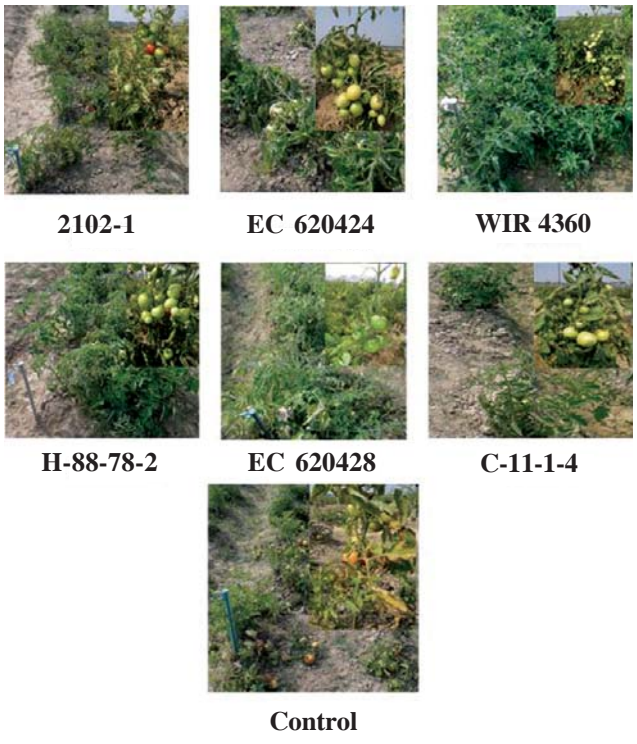

Fig. 53. Tomato lines showed resistance against *Fusarium* wilt under field conditions

d. DISEASE MANAGEMENT

Talc based formulation of *Trichoderma* isolates (Phyto 1-15), two fungicides and botanicals (datura and garlic extracts) were evaluated against *Fusarium* wilt of tomato under field conditions and all the treatments were found effective in controlling the *Fusarium* wilt. However, Phyto 14, Phyto 9 and Phyto 11 showed superiority over other isolates (Fig. 54, Table 28).The botanical extracts of datura and garlic performed better.

Table 27. Screening of tomato germplasm lines for resistance against wilt disease

Reaction	No. of lines	Germplasm lines
Resistant	7	2102-10-1, EC-620424, WIR- 4360, EC-620428, 2103-1-2, H-88-78-2, C-11-1-4
Moderately resistant	5	FEB-2, EC-520060, A-15-6-1, A-15-9-1, C-14-5-3,
Susceptible	3	WIR-5032, Hawai- 3998, DVRT-1

Table 28. Effect of biocontrol agents, botanicals and chemicals on *Fusarium* wilt and yield of tomato under field conditions

Treatment	Wilt incidence (%)*	Yield (Q/ha)**
Phtyo-1	22.1	206.25
Phyto-2	22.1	220.00
Phyto-3	21.3	200.00
Phyto-4	18.8	205.00
Phyto-5	16.6	165.00
Phyto-6	20.0	161.67
Phyto-7	15.0	210.00
Phyto-8	14.5	212.50
Phyto-9	12.1	185.83
Phyto-10	15.6	187.92
Phyto-11	13.8	239.58
Phyto-12	14.8	198.33
Phyto-13	18.3	187.67
Phyto-14	12.5	176.67
Phyto-15	17.5	193.33
Carbendazim+mancozeb	18.8	175.83
Carbendazim	14.0	168.33
Datura extract	14.5	177.50
Garlic extract	11.6	208.33
Control	38.8	142.50
CD (5%)	4.3	13.0

*Values were arc sine transformed before the analysis,
**Yield upto 4 harvest

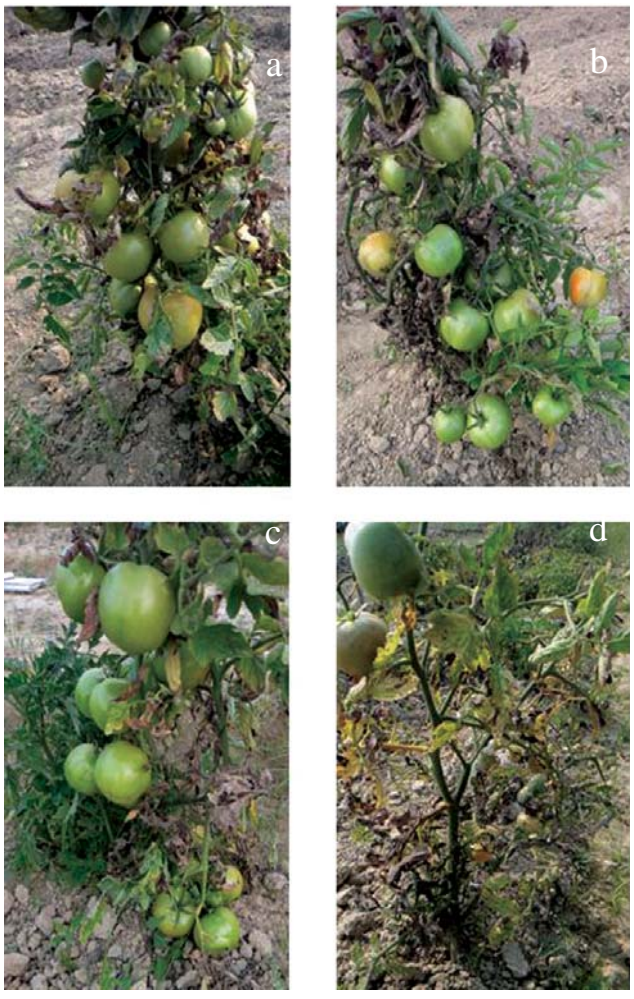

Fig. 54. Effect of *Trichoderma* isolates on *Fusarium* wilt of tomato under field conditions. a - Phyto 14; b - Phyto 11; c - Phyto 9; d - control

NATIONAL RESEARCH CENTRE FOR BANANA, TIRUCHIRAPALLY

Principal Investigator:
Dr. R. Thangavelu

Co-Investigator:
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a. DISEASE MANAGEMENT

Field evaluation of endophytic and rhizospheric bacterial and fungal isolates for the suppression of *Fusarium* wilt disease in cv. Grand Naine

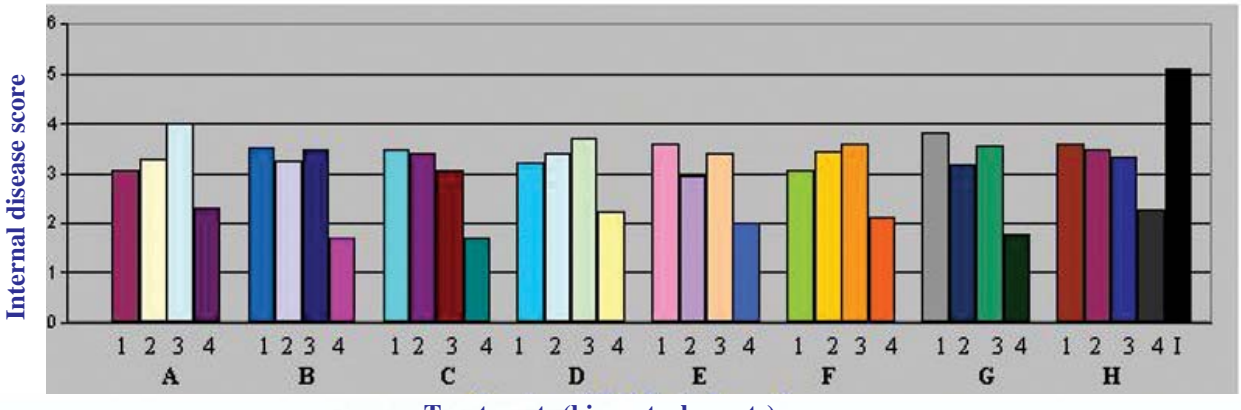
Among the different endophytic and rhizospheric fungal and bacterial isolates for the suppression of *Fusarium* wilt disease in cv. Grand Naine, the combined application of fungal endophytic *Penicillium pinophilum* Bc2 + rhizospheric *T. koningii*, endophytic *Penicillium* spp. Dsr1 + rhizospheric *T. koningii*, and bacterial endophytic Tvpr1 + rhizospheric Jrb1 have significantly decreased the *Fusarium* wilt disease severity with the disease score of 1.70, 1.68, and 1.76, respectively, compared to the untreated plants. The application of these strains also increased the bunch weight (26.24%) and number of hands (12.88%) significantly as compared to untreated control plants. Besides, the number of plants harvested was 94.4% whereas in the untreated control it was only 52.63% (Fig. 55).

Evaluation of fungicide resistant mutant of *Trichoderma* spp. isolates along with chemical fungicide

The fungicide resistant mutant of *Trichoderma* spp. isolates *viz.,* endophytic *T. harzianum* Prr2, *P. pinophylum* Bc2 and *Penicillium* sp. isolates and rhizospheric *T. harzianum* and *T. koningii* isolates were evaluated along with chemical fungicide Difenaconazole (0.1%) @ 250 ml/ plant for the suppression of *Fusarium* wilt disease in cv. Grand Naine. The combined application of rhizospheric and endophytic fungal antagonists along with or without fungicide application significantly increased the bunch weight (up to 74.8%) and suppressed the *Fusarium* wilt disease compared to untreated plants (Fig. 56).

Pot culture evaluation on the combined application of endophytic fungi (biopriming) and botanical leaf extracts against *Fusarium* wilt pathogen *Foc* - VCG-0124

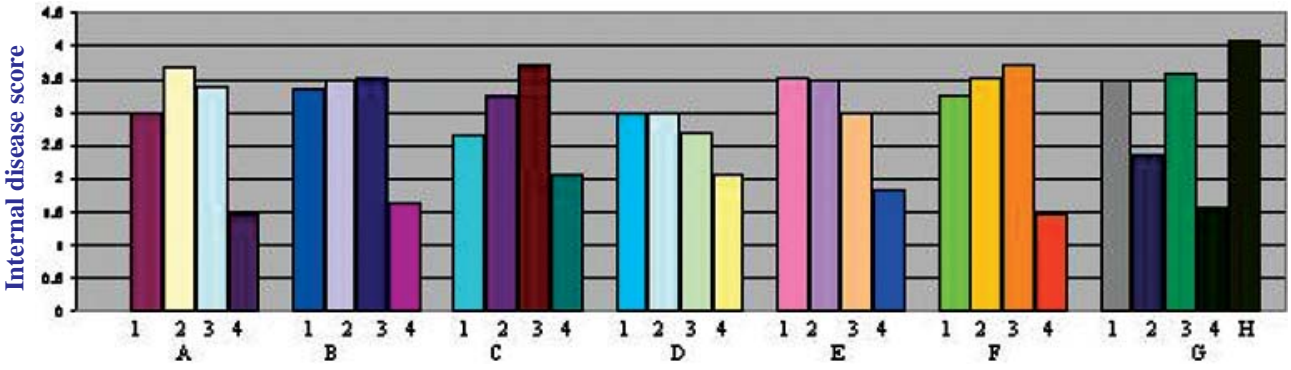
The combined application of three effective



A. Endo. *T. harzianum* (Prr2) + Rhizo. *T. longibrachiatum*
B. Endo. *P. pinophilum* (Bc2) + Rhizo. *T. asperellum*
C. Endo. *Penicillium* sp (DSr1) + Rhizo. *T. asperellum*
D. Endo. *Pseudomonas putida* (C4R4) + Rhizo. *Bacillus* sp (Jrb1)
E. Endo. *Acrhomobacter* sp (GCr1) + *Bacillus cereus* (Jrb5)
F. Endo. *Rhizobium* sp (LPr2) + *Bacillus* sp (Jrb1)
G. Endo. *Bacillus* sp (TVPr1) + Rhizo. *Bacillus* sp (Jrb1)
H. Endo. *Bacillus* sp (TVPr1) + Rhizo. *Pseudomonas* sp (Jrb2)
I. Control

Methods of application
1. at the time of planting
2. 2nd MAP-drenching
3. 4th MAP-drenching
4. at planting + 2nd + 4th MAP

Fig. 55. Field evaluation of endophytic and rhizospheric bacterial and fungal isolates for the suppression of *Fusarium* wilt disease in cv. Grand Naine



A. Mut. Endo. *T. harzianum* (Prr2) + Mut . Rhizo. *T. longibrachiatum*
B. Mut. Endo. *P. pinophilum* (Bc2) + Mut. Rhizo. *T. asperellum*
C. Mut. Endo. *P enicillium* sp (DSr1) + Mut.Rhizo. *T. asperellum*
D. Difenaconazole (0.1%)
E. Mut. Endo. *T. harzianum* (Prr2) + Rhi. *T. harziaum* + Difenaconazole (0.1%)
F. Mut . Endo. *P. pinophilum* (Bc2) + Mut. *T. asperellum* + Difenaconazole(0.1%)
G. Mut. Endo. *Penicillium* sp (Dsr1) + Mut. *T. asperellum* + Difenaconazole(0.1%)
H. Control

Methods of application

1. at the time of planting,
2. 2nd MAP-drenching,
3. 4th MAP-drenching,
4. at planting+2nd+4th MAP

Fig. 56. Field evaluation of fungicide resistant mutant of *Trichoderma* spp. isolates along with chemical fungicide Difenaconazole for the management of Foc

endopyhtic fungi viz., *T. harzianum* (Prr2), *P. pinophilum* (Bc2), *Penicillium* spp. (Dsr1) @ and three effective botanicals *viz.*, *Alpinia* sp., *Hibiscus* sp. and Zimmu in cv. Grand Naine, significantly increased the plant growth parameters such as height (33.60%), girth (80%), number of leaves (42.11%), leaf area (128.15%) and number of roots (143.04) when compared to *Foc* inoculated control. Complete control (100% reduction) of the disease was observed in *T. harzianum* (Prr2) + *Hibiscus* sp., *T. harzianum* (Prr2) + Zimmu, *P. pinophilum* (Bc2) + *Alpinia* sp., *P. pinophilum* (Bc2) + *Hibiscus* sp., *P. pinophilum* (Bc2) + Zimmu, *Penicillium* (Dsr1) + Zimmu combination (Fig. 57, Table 29).

Pot culture evaluation of endophytic bacteria (biopriming) + botanical leaf extract against *Foc* VCG-0124

The combined application of three effective endopyhtic bacteria viz., *Pseudomonas putid*a (C4r4), *Achromobacter* sp. and *Bacillus* sp and three effective botanicals viz., *Alpinia* sp., *Hibiscus* sp. and Zimmu against *Fusarium* wilt pathogen (*Foc*-VCG 0124) under pot culture condition in cv. Grand Naine, significantly increased the plant growth parameters such as height (up to 38.3%), girth (up to 71.4%), number of leaves (42.1%), leaf

Fig. 57. Combined effect of *Penicillium pinophilum* (BC2) + Zimmu

area (93.5%) and number of roots (143.04) when compared to *Foc* alone inoculated control plants. Complete control (100% reduction) of the disease was observed in *P. putida* + Alpinia, *P. putida* + *Hibiscus* sp., *P. putida* + Zimmu, *Bacillus* sp. + Zimmu combinations (Table 30).

Development of liquid formulation for the effective *Trichoderma* spp. isolates

The population of *Trichoderma* spp. was 1.66 x10^{10} in molasses at 15th month of sampling in liquid formulation. The efficacy of liquid formulation of *Trichoderma longibrachyatum* (which was stored for 13 months at 25 ± 2°C) at 5, 10 and 15% conc was tested in cv. Grand Naine. It was found effective in controlling external and internal symptoms of Fusarium wilt disease even after six months of planting (Table 31).

Table 29. Pot culture evaluation of endophytic fungi (bio priming) and botanical leaf extract against *Foc* VCG-0124

Treatment	Growth parameters				Total number of roots	Internal score (1-6 scale)
	Plant height (cm)	Girth (cm)	Total number of leaves	Leaf area (cm^2)		
Endophytic *T. harzianum* (Prr2) + *Alpinia* sp.	24.6 (21.7)	7.8 (56)	4.6 (21.0)	344.2 (125.3)	23.4 (48.1)	1.4
Endophytic *T. harzianum* (Prr2) + *Hibiscus* sp.	26.0 (28.7)	7.6 (52)	5.0 (31.5)	341.1 (123.3)	26.2 (65.8)	1.0
Endophytic *T. harzianum* (Prr2) + Zimmu	24.0 (18.81)	6.5 (30)	4.8 (26.32)	284.10 (85.9)	26.5 (67.7)	1.0
Endophytic *P. pinophilum* (Bc2) + *Alpinia* sp.	23.2 (14.8)	5.2 (4)	4.2 (10.5)	265.7 (73.9)	23.8 (50.6)	1.0
Endophytic *P. pinophilum* (Bc2) + *Hibiscus* sp.	27.0 (33.66)	6.6 (32)	5.0 (31.5)	273.97 (79.3)	24.4 (54.43)	1.0
Endophytic *P. pinophilum* (Bc2) + Zimmu	26.2 (29.7)	9.0 (80)	5.2 (36.84)	348.5 (128.1)	30.6 (93.6)	1.0
Endophytic *Penicillium* spp. (Dsr1) + *Alpinia* sp.	27.0 (33.6)	7.0 (40)	5.4 (42.1)	349.4 (128.7)	28.0 (77.22)	1.4
Endophytic *Penicillium* spp. (Dsr1) + *Hibiscus* sp.	26.0 (28.7)	6.0 (20)	5.4 (42.11)	320.4 (109.7)	38.4 (143.04)	2.0
Endophytic *Penicillium* spp. (Dsr1) + Zimmu	26.0 (28.7)	6.8 (36)	4.6 (21.0)	315.3 (106.4)	28.6 (81.01)	1.0
Control	21.5 (6.44)	6.5 (30)	4.0 (5.26)	315.9 (106.7)	22.3 (41.14)	1.0
Foc alone	20.2 (0.0)	5.0 0.0)	3.8 (0.0)	152.7 (0.0)	15.8 (0.00)	4.6
CD (P=0.05%)	5.2	2.3	1.4	5.2	6.2	0.3

Endophytic tissue colonization and quantification of rhizospheric *Trichoderma* spp. population

The colonization of root, corm and stem of banana tissues by the *T. harzianum* of endophytic origin and *T. longibrachiatum* of rhizospheric origin, was found only on the root and corm tissues and not the stem tissues (Fig. 58 and 59). However, the population of *T. longibrachiatum* isolated from the rhizosphere was significantly higher in the rhizosphere soil compared to *T. harzianum* isolated from the plant tissues.

Quantification of rifampicin mutant of endophytic and rhizospheric bacterial isolates in sterile soil

The quantification of rifampicin mutants *Pseudomonas putida* (C4r4), *Achromobacter* sp (Gcr1), R*hizobium* sp (Lpr2) and *Bacillus* sp (Tvpr1) was done.

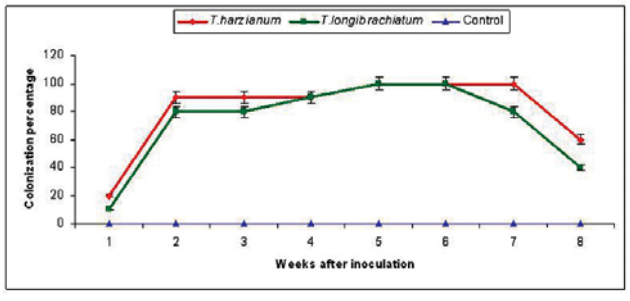


Fig. 58. Colonization of *Trichoderma* sp. in banana corm

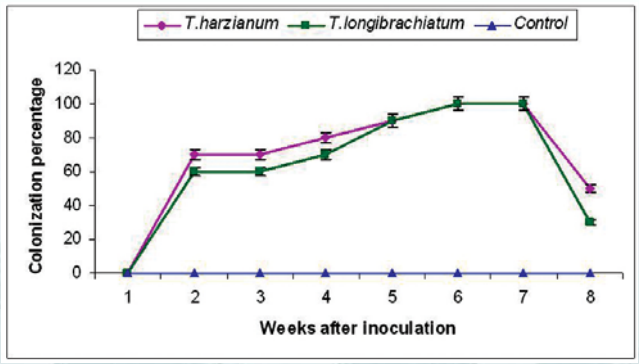


Fig. 59. Colonization of *Trichoderma* sp. in banana root

Table 30. Pot culture evaluation of endophytic bacteria (biopriming) and botanical leaf extract against *Foc* VCG-0124

Treatment	Percent increase of growth parameters				Total number of roots	Internal score (1-6 scale)
	Height (cm)	Girth (cm)	Total number of leaves	Leaf area (cm^2)		
Pseudomonas putida (C4r4) + *Alpinia* sp.	7.4	33.9	39.4	21.2	98.8	1.0
Pseudomonas putida (C4r4)+ *Hibiscus s*p.	18.6	42.8	31.5	53.6	110.0	1.0
Pseudomonas putida (C4r4) + *Zimmu*	31.7	39.2	26.3	49.7	141.1	1.0
Achromobacter sp. (Gcr1) + *Alpinia* sp.	27.1	64.2	36.8	62.5	100.0	1.4
Achromobacter sp. (Gcr1) + *Hibiscus* sp.	38.3	50.0	36.8	80.4	76.6	2.2
Achromobacter sp. (Gcr1) + *Zimmu*	30.8	21.4	10.5	44.1	68.8	1.0
Bacillus sp.(Tvpr1) + *Alpinia* sp.	26.1	55.3	18.4	58.1	86.1	1.25
Bacillus sp.(Tvpr1) + *Hibiscus* sp.	17.7	28.5	21.0	31.0	51.1	2.2
Bacillus sp. (Tvpr1) + *Zimmu*	33.6	71.4	42.1	93.5	77.7	1.0
Control	1.4	3.5	10.5	19.8	57.7	1.0
Foc alone	0.0	0.0	0.0	0.0	0.0	4.8
CD (P=0.05%)	6.5	2.4	1.3	3.1	0.8	0.4

Table 31. Efficacy of liquid formulation (rhizospheric *T. asperellum*) against *Foc* VCG-0124

Treatment	Growth parameters				Total number of roots	Internal score (1-6 scale)
	Height (cm)	Girth (cm)	Total number of leaves	Leaf area (cm^2)		
Endophytic *T. harzianum*	24.6 (21.7)	7.8 (56)	4.6 (21.0)	344.2 (125.3)	23.4 (48.1)	1.4
Rice chaffy grain formulation of *T. asperellum* @ 30 g/plant	21.75 (19.8)	6.3 (34.04)	6.0 (30.43)	3631.8 (222)	25.2 (54.6)	1.0
Liquid formulation 5 %	19.0 (4.11)	6.0 (27.66)	6.0 (30.43)	1221(8.14)	26.66 (63)	1.0
Liquid formulation 10%	22.0 (0.55)	6.0 (7.66)	5.7 (23.91)	3071.72 (172)	21.75 (33)	1.0
Liquid formulation (15%)	23.2 (27.12)	6.3 (34.04)	6.2 (34.78)	2573.5 (128)	21.75 (33)	1.0
Foc alone	18.25 (0)	4.7 (0)	4.6 (0)	1129.14 (0)	16.33 (0)	4.5

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS, MAU

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a. *FUSARIUM* DIVERSITY

Computational mining and genome wide distribution of microsatellite in *Fusarium oxysporum* f. sp. *lycopersici*

Total genome sequence data (59.9 Mb) of *F. oxysporum f. sp. lycopersici* was assembled into 423 scaffolds and used to explore mono-, di-, tri-, tetra-, penta- and hexa-nucle-otide motifs with a repeat of 6 times. A total 13864 SSRs were identified from whole genome data of *F. oxysporum* f. sp. *lycopersici*. The relative abundance and density of SSRs were 231.45 SSR/Mb and 2643.73 bp/Mb, respectively.

Microsatellite distribution on chromosome

Chromosome 1 possessed highest number of SSRs (7008) and chromosome 13 had the least number of SSRs (119). Four hundred and twenty three SSRs were identified in the contigs not mapped to any chromosome. Tetra-nucleotides repeats were the most abundant (3837) repeats in all the chromosomes accounting 27.67% of SSRs. Maximum relative abundance (1023 SSR/Mb) and density of SSRs (114.46 bp/Mb) was occurred on chromosome 1 and followed by chromosome 2 (148 SSR/Mb, 18.1 bp/Mb), chromosome 7 (147 SSR/Mb, 16.55 bp/Mb) , chromosome 8 (146 SSR/Mb, 17.17 bp/Mb), chromosome 15 (145 SSR/Mb, 18.6 bp/Mb) and 14 (144 SSR/Mb, 21.6 bp/Mb), respectively (Tab. 3). Least relative abundance of repeat motifs were occurred on chromosome 12 (68 SSR/Mb) and 13 (68 SSR/Mb), while least density was observed on chromosome 11 (7.40 bp/Mb) and 12 (7.41 bp/Mb), respectively (Table 32).

Table 32. Distribution of SSRs in different chromosomes identified from public available whole genome database of *F. oxysporum* f. sp. *lycopersici*

Chromosome	Mono	Di	Tri	Tetra	Penta	Hexa	Size (Mb)	SSR (Mb)	bp (Mb)	SSR
1	1102	296	1777	1802	1358	673	6.85	1023	114.46	7008
2	140	35	216	176	176	88	5.58	148	18.1	831
3	204	19	128	202	96	40	5.63	122	13.23	689
4	110	21	155	148	86	52	5.21	109	12.86	572
5	91	25	160	231	119	72	4.91	142	17.07	698
6	128	12	96	158	85	34	4.59	111	11.95	513
7	99	25	153	180	126	60	4.35	147	16.55	643
8	92	15	139	165	105	67	3.98	146	17.17	583
9	65	18	110	122	89	38	3.3	133	17.22	442
10	75	10	97	128	63	36	2.9	141	21.59	409
11	30	4	56	59	19	9	2.34	75	7.402	177
12	26	10	43	46	19	8	2.23	68	7.41	152
13	10	2	44	36	14	13	1.75	68	8.86	119
14	36	10	45	99	45	17	1.65	144	21.6	252
15	63	8	75	147	41	19	2.43	145	18.6	353
Scaffold	100	12	73	138	68	32	3.75	112	7.592	423
Total	2371	522	3367	3837	2509	1258	59.1	234	2643.73	13864

Characterization of *Fusarium* isolates through multilocus sequence typing

The multilocus sequence typing of different *Fusarium* species were undertaken. Cultures received from different centres were used for MLST using various conserved and housekeeping genes like ITS, beta-tubulin, transcription elongation factor alpha and mitochondrial gene. Twenty isolates *F. oxysporum* f. sp. *ciceri* were used for amplification of above mentioned genes. All resulted in the amplification of desired size of amplicon. Out of these amplified genes, ITS and beta-tubilin genes were sequenced. Phylogenetic analysis was done by constructing the dendogram with boot strap value (Fig. 60 and 61).

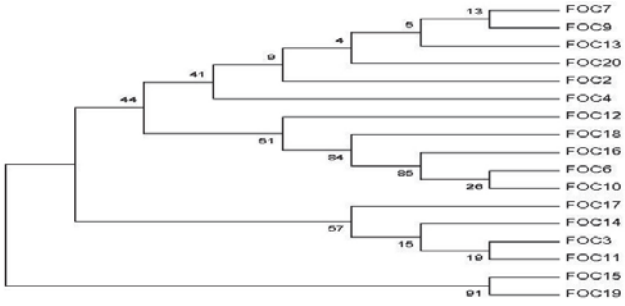


Fig. 60. Dendogram on the basis of ITS sequence of *F. oxysporum* f. sp. *ciceri*

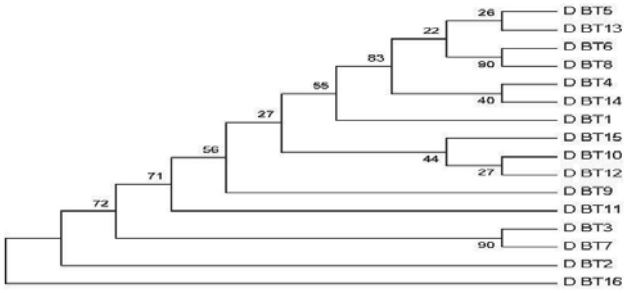


Fig. 61. Dendogram on the basis of beta-tubulin sequence of *F. oxysporum* f. sp. *ciceri*

Protein profiling of different isolates of *Fusarium* spp.

To study the variability through protein profiling, total protein from *F. udum* was extracted. The isolated protein range was from the ~25kDa to ~180kDa (Fig. 62).

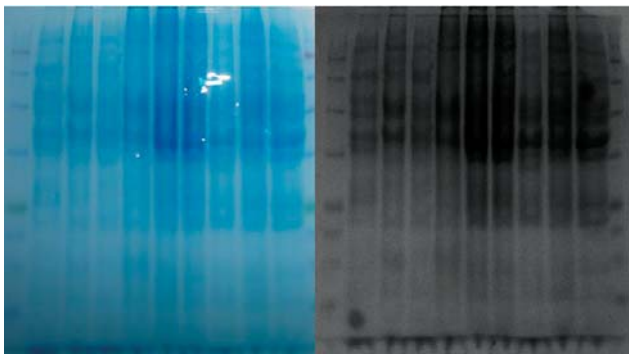
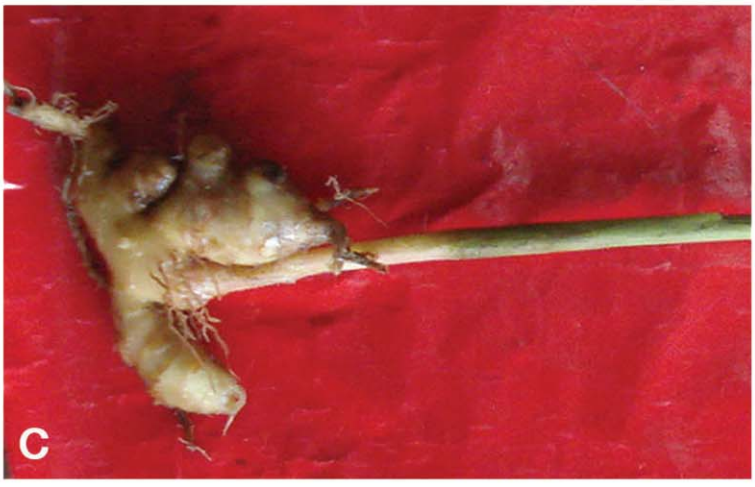


Fig. 62. Total protein profiling of *F. udum*

PhytoFuRa

Ralstonia



Symptoms of bacterial wilt A. Brinjal, B. Ginger, C. Tomato, D. Chilli

INDIAN INSTITUTE OF SPICES RESEARCH, KOZHIKODE

Principal Investigator:
Dr. D. Prasath

Co-investigator:
Dr. R. Suseela Bhai

a. DIAGNOSTICS

Development Race4/biovar 3 specific primers for *R. solanacearum* infecting ginger

Eight primers were designed from the *recN* and *gyrB* sequences of *R. solanacearum* and amplified. None of them were found to be specific to Race 4 Biovar 3 of ginger; the primers amplified all the biovar 3 strains.

b. HOST RESISTANCE

Curcuma amada Roxb. is a potential donor for bacterial wilt resistance to *Zingiber officinale,* if the exact mechanism of resistance is understood.

Isolation and characterization of NBS-LRR resistance gene analogs in *C. amada*

The degenerate R-gene primers (Kanazin *et al.* 1996; Nair and Thomas 2007) amplified a common band of ~ 500 bp from the genomic DNA of *C. amada* and *Z. officinale*. The eluted bands when sequenced resulted in product size of 531 and 534 nucleotides for *C. amada* and *Z. officinale* respectively. Cluster analysis of the amino acid sequences of R-genes of *C. amada*, Z. *officinale,* and other plant species showed that the NBS analogs belonged to non-TIR-NBS-LRR subfamily with highly conserved tryptophan (W) as the last residue of kinase-2 domain.

There was significant sequence similarity to the Kinase-1a, Kinase-2 and hydrophobic GLPL motifs of the NBS domain of the known genes. The sequences of *Z. officinale* and *C. amada* showed 28% similarity to each other. *Z. officinale* showed greater similarity to *Z. zerumbet* (88%), *Manihot esculenta* (44%), *Oryza sativa Japonica Group* (43%), *Elaeis guinensis* (41%), *Vitis vinifera* (42%), *Gossypium hirsutum* (37%), *Arabidopsis thaliana* (42%), *Medicago ruthenica* (39%) and *Malus baccata* (42%). *C. amada* showed greater similarity to *Hordeum vulgare* (55%), *Brachypodium distachyon* (51%), *and Sorghum bicolor* (47%), *Saccharum officinarum* (45%), *and Prunus persica* (44%). Phylogenetic tree constructed using neighbour joining method to visualize the relative distance of the *C. amada* and *Z. officinale* sequences to R-genes and RGCs from other species revealed that *Z. officinale* and *C. amada* belonged to two separate clusters (Fig. 63). *Z. officinale* R-gene analogs are different from *C. amada*. The expression of the RGA was significantly higher for *C. amada* compared to *Z. officinale*. R-gene was readily induced by the bacterium in mango ginger, while its induction was very weak and slow in ginger. The expression peaked up 24 hours after inoculation in *C. amada* (Fig. 64). These results suggest that the R-genes possibly play a role in the molecular defense response of mango ginger to pathogen attack.

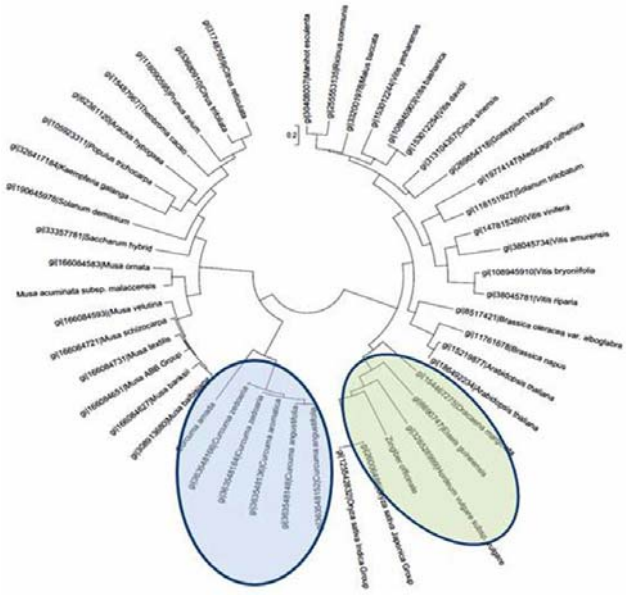


Fig. 63. Phylogenetic relationships of the *Z. officinale* and *C.amada* RGCs with other plant species

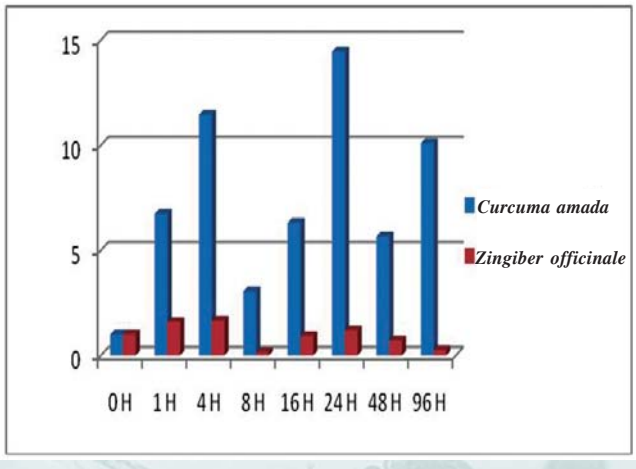


Fig. 64. Time course of the expression levels of the Rca and Rzo in *C. amada* and *Z. officinale* determined by qPCR after *R. solanacearum* inoculation

c. HOST PATHOGEN INTERACTION

Analysis of differentially expressed genes in *C. amada and Z. officinale* upon infection with *R. solanacearum* by suppression subtractive hybridization (SSH)

RNA samples derived from plants after 4 and 8 hai was used to understand the early genes involved in bacterial wilt resistance in *C.amada* by subtractive hybridization. The subtracted products on gel analysis appeared as a smear ranging in size from 300 bp -1kb with 2 to 4 definite bands clearly distinguishing them from the un-subtracted sample control. The subtracted skeletal muscle sample showed DNA fragments corresponding to the *f*X174/*Hae*III digest. The control-subtracted sample provided in the kit gave an identical pattern, which confirms the efficiency of the subtraction experiment.

A forward SSH library containing transcripts differentially expressed in *C. amada* infected by *R. solanacearum* was constructed (350 colonies) using RNA from *C. amada* as a tester and RNA from *Z. officinale* as driver. Randomly selected subtracted clones were PCR-amplified using the adaptor primer pair of the subtraction. Plasmid DNAs of 150 recombinant white clones were purified and subjected to sequence determination.

Computational analysis of subtracted transcriptome of *C. amada*

Clones containing differentially expressed cDNAs of *C. amada* were sequenced using M13 forward and reverse primers. Of the 150 clones sequenced, 138 produced suitable sequences and were evaluated by computational analysis. All the sequences were annotated using the PSI-BLAST program. Of the 138 SSH sequences, sequences coding for putative proteins related to defense response during *C. amada* - *R. solanacearum* interaction (high homology between 85 and 100% identity) were, glutathione -S- transferase (GST), putative cytosolic ascorbate peroxidase (APX), cell wall associated hydrolase, xyloglucan transglycosylase (XTH), cytochrome p 450, metallocarboxypeptidase inhibitor, peroxiredoxin and thioredoxin dependent peroxidase. Fifteen sequences had no identity with sequences in the databases.

To understand comprehensive overview of *C. amada* subtracted transcriptome, associated with its molecular function, biological process, cellular component and gene ontology annotation was implemented with Blast2GO tool. The other 125 SSH sequences showed high homology with the genes whose function were known and they were classiûed into three categories based on gene ontology terms, proteins involved in biological processes (31%), cellular component (43%) and molecular function (26%).

Transcriptome analysis

mRNA-Seq profiling of *C. amada* and *Z. officinale* leaves following infection with *R. solanacearum* over a 72 h period was done. Leaf samples were collected, pooled and RNA was isolated. Using an Illumina paired-end sequencing platform a total of 31,845,321x2 (101 base), 24,107,482x2 (101 base) raw reads were generated, accounting for approximately 6.43Gb and 4.87Gb of sequence data for *C. amada* and *Z. officinale* respectively.

Table 33. Overview of the sequencing and *de novo* assembly

Properties	*C. amada*	*Z. officinale*
Total nucleotides (Nt)	3,216,377,421	2,434,855,682
Total number of clean reads	29875247	24107482
Q20 percentage	93.81	81.85
GC precentage	47	47
Total number of contigs	132714	104870
Total length of contigs (Nt)	106,354,905	77,668,629
Mean length of cotigs (Nt)	801.38	740.61
N50 of contigs	1392	1229
Smallest contig length	201	201
Largest contig length	28483	17415
Total number of unigenes	79018	63765
Distinct clusters	101049	85910
Distinct singletons	82469	73739

Transcriptome assembly

De-novo transcriptome assembly was performed using trinity assembler, with the default settings *k*-mer size of 25, minimum contig length of 200, paired fragment length of 500, 16 CPUs, with butterfly Heap space of 100G (allocated memory),

which generated 132,714 and 104,870 contigs, with average lengths of 801.38 and 740.61 bp, from the mango ginger and ginger libraries, respectively (Table 33). Further transcriptome assembly using Bowtie resulted in 45045 and 65535 transcripts (Table 34). Fig.65 shows the transcript length distribution ranging from 200 bases to more than 6000 bases.

Trinity *de novo* assembled transcripts of CA were taken as a reference sequence against which both ZO and CA raw reads were mapped using Tophat software version 2.0.4. The mapped data was run through Cufflinks, Cuffcompare, and Cuffdiff and CummeRbund pipeline to get the list of differentially expressed genes. Cuffdiff was used to generate a differential expression result which was further used to plot Heatmap and differential expression plots by CummeRbund package. After filtering out the list of differentially expressed genes, the result was further filtered on including only the disease related genes along with their corresponding FPKM values (Fig. 66).

Table 34. Assembly summary of *C. amada* (CA) and *Z. officinale* (ZO)

Properties	*C. amada*	*Z. officinale*
No. of transcripts	45046	65536
Maximum transcript length	17415	28483
Minimum transcript length	200	200
Total transcript length (bases)	48,132,098	73,069,608
Mean transcript length	1068.53	1114.91

Deciphering the biochemical basis of resistance in mango ginger against bacterial wilt

Unraveling the resistance mechanism by genetic and biochemical analysis can make *C. amada*, a potential source of resistance. Biochemical analysis was carried out to explore the potential of *C. amada* as an antimicrobial agent. Volatile oil and three solvent extracts (hexane, methanol and chloroform) from the rhizome of *C. amada* were used to examine the antimicrobial activity *in-vitro*. Volatile oil was

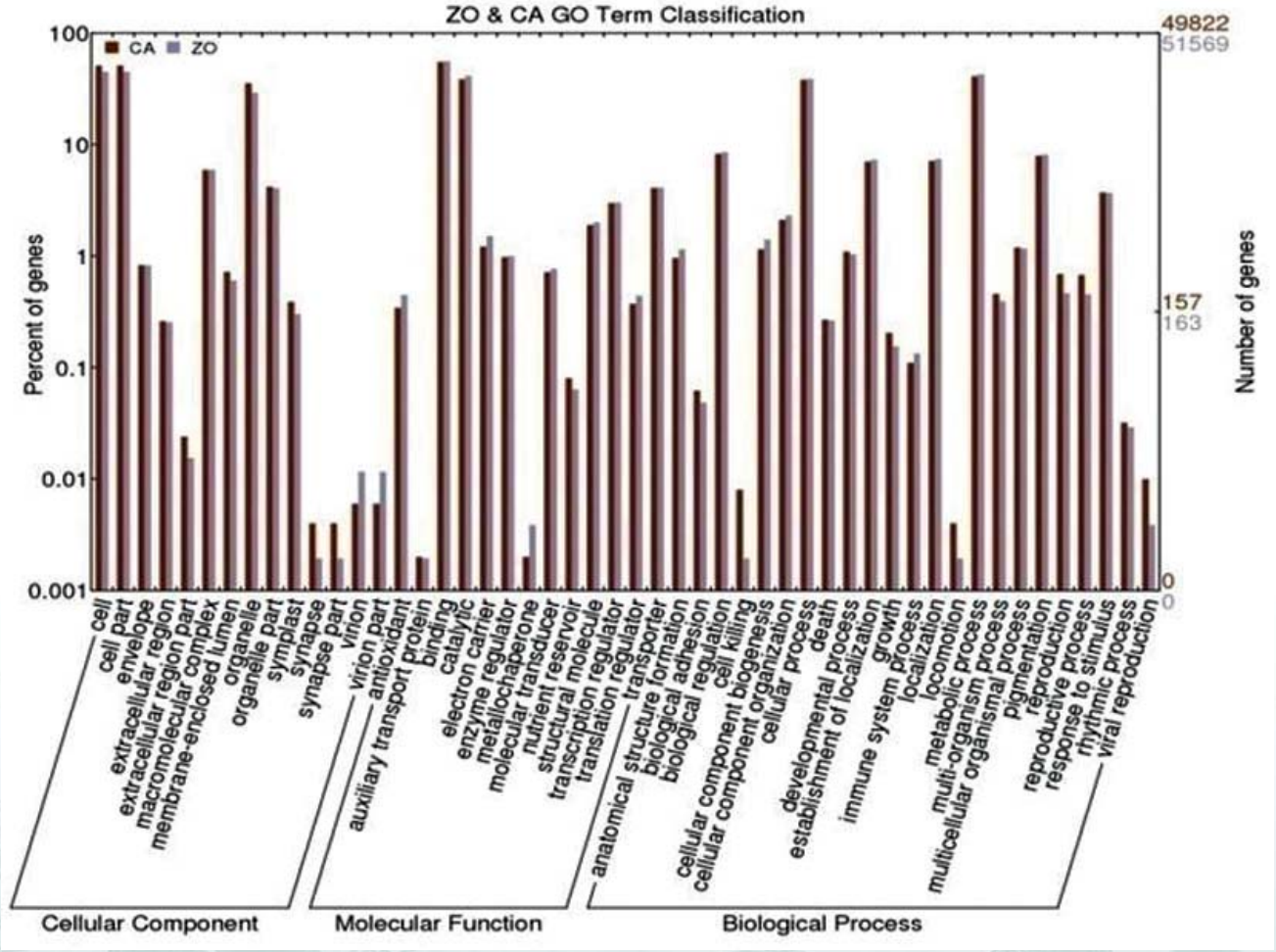


Fig. 65. Histogram presentation of Gene Ontology classification of putative molecular functions of unigenes from mango ginger and ginger tissues and biological processes in which they are involved

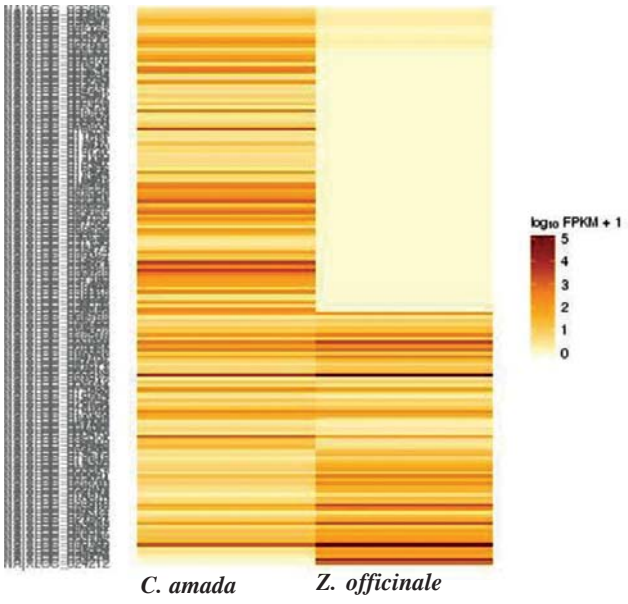


Fig. 66. Heatmap plotted for defense related genes

extracted through hydro distillation and solvent extracts were obtained by sequential cold extraction with hexane, chloroform and methanol in the order of their increasing polarity. Among the solvent extracts chloroform extract exhibited greatest antibacterial activity against *R. solanacearum*, followed by hexane and methanol extracts. Methanol extract exhibited least effect. Essential oil at 1.5% (v/v) caused the complete death of bacteria showing greatest antimicrobial effect (Fig. 67). The study further demonstrated that the active compound against phytopathogens concentrated in the volatile oils. Essential oil was subjected to GC-MS analysis and the major components detected were β-Myrcene and β-Pinene (Table 35)**.** Further β-myrcene and beta β -pinene standards were tested for their inhibitory activity against *R. solanacearum*. Both of the components showed antibacterial activity with myrcene showing greater activity.

Table 35. Volatile constituents (%) of *C. amada* essential oil

Constituent	Area %
β-Myrcene	38.00
β -pinene	10.28
Perillene	3.74
Caryophyllene oxide	2.65
Camphene	1.98
Cis-ocimene	1.77
D-limonene	0.94
β Terpineol	0.91
E-Caryophyllene	0.90
n-Hexadecanoic acid	0.72
1, 8-Cineole	0.65
Camphene	0.64
Ar-Tumerone	0.52
Linalool	0.46
Geranial	0.29
2-Nonanone	0.38
Trans-ocimene	0.33
Cymene	0.25
Trans-Pinocarveol	0.25
Myrtenal	0.21
Borneol	0.21
Myrtenol	0.20
Perillyl alcohol	0.20
z-Citral	0.17
Curlone	0.16
Tumerone	0.16

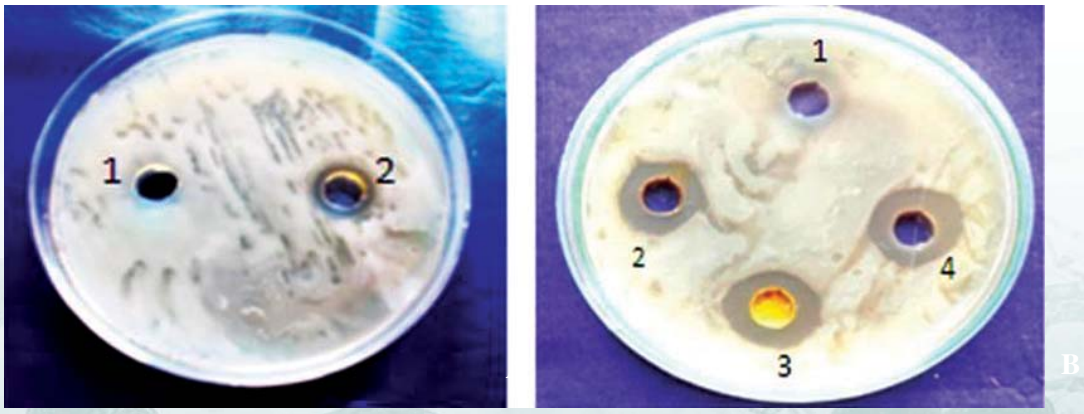


Fig. 67. A. Effect of essential oil of *C. amada* on *R. solanacearum* 1) Control, 2) 1% oil; B. Inhibitory activity of solvent extracts on *R. solanacearum* 1) Control, 2) Hexane extract, 3) Chloroform extract, 4) Methanol extract

d. DISEASE MANAGEMENT

Isolation and characterization of phages infecting *R. solanacearum*

The phages were isolated from rhizosphere soil samples collected from ginger growing areas of Kerala and Karnataka by filtering the soil suspension using 0.45µm membrane filter and soft agar overlaying on CPG agar contacting *R. solanacearum* as the host. A phage could be isolated from ginger rhizosphere soil collected from Kumichi (Pulpally) of Wayanad District. The host range of this phage was checked using seventeen different isolates of *R. solanacearum* collected from different locations (Fig. 68, Table 36). This phage was found to be infecting all the isolates collected from Wayanad and one isolate collected from Thamarasserry. The phage was PEG purified and titrated. The stock contains 10^7 pfu/ml.

Testing the biocontrol potential of phages

Genomic DNA was extracted from the phage isolated from Wayanad. 750µl of PEG purified stock of phage was used for DNA extraction. Presence of clear plaques and absence of attP region indicates the lytic nature of the phage which can be used as a tool to identify the biocontrol nature of the phage. Since the isolated phage showed clear plaques with *R. solanacearum* isolate, attempt was made to amplify the attP region in order to confirm the biocontrol nature of the isolated phage. The phage DNA attP site was amplified using the primer pairs attP - L 5'-CAGTATGTGTCCTGGGTGTTTGTCTACCG-3' and attP - R 5'-CTCTTATCAGAACGCCCCACCTCCC-3'. No

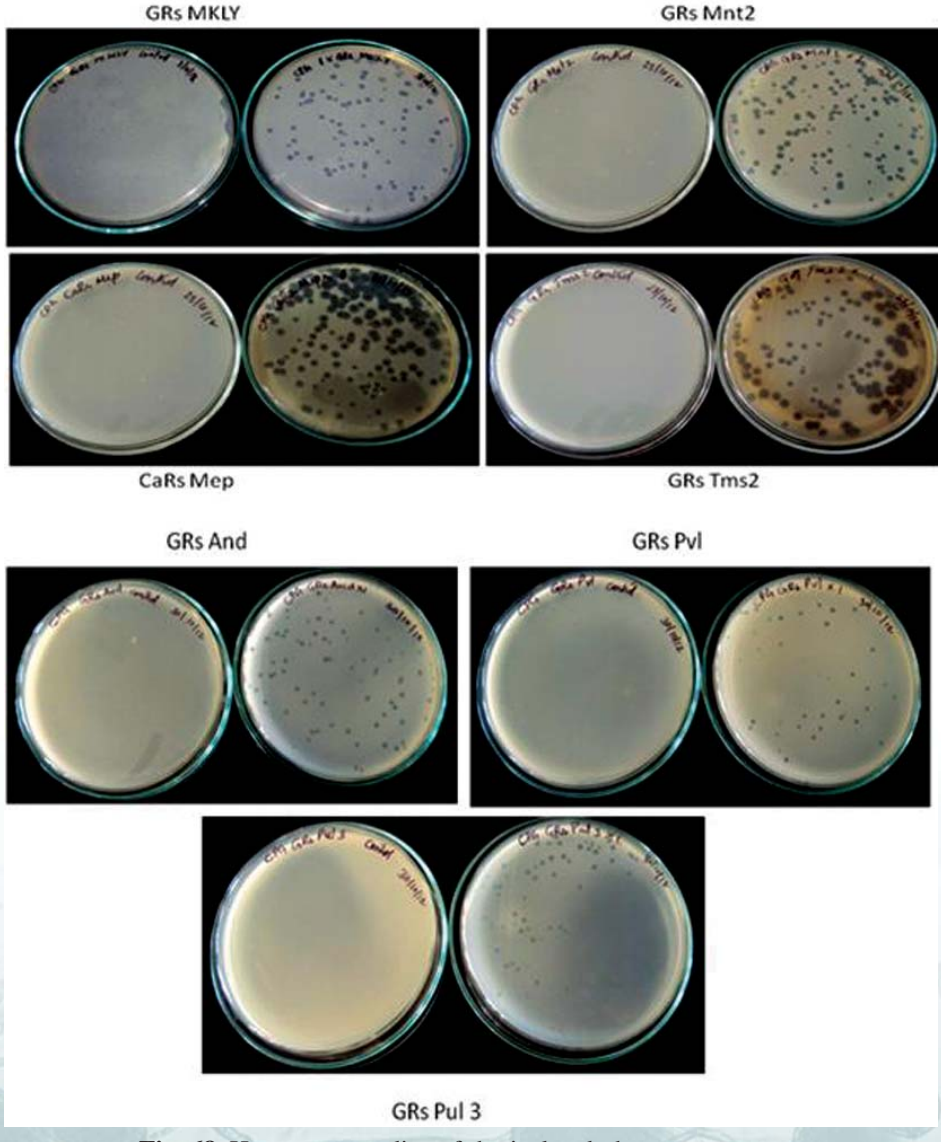


Fig. 68. Host range studies of the isolated phages

Table 36. Host range of isolated phages

Isolate	Place of collection	Host crop	Lysis by phage infection
GRs MKLY	Mullankolly (Wayanad)	Ginger	+
GRs Mnt2	Mananthavadi (Wayanad)	Ginger	+
CaRs Mep	Meppadi (Wayanad)	Cardamom	+
GRs Tms2	Thamarassery (Kozhikode)	Ginger	+
GRs And	Andoor (Wayanad)	Ginger	+
GRs Pvl	Palavayal (Wayanad)	Ginger	+
GRs Pul3	Pulpally (Wayanad)	Ginger	+
GRs Pkd	Muthalamada (Palakkad)	Ginger	_
GRs Ktm	Kothamangalam	Ginger	_
UTT 5	Uttarakhand	Tomato	_
UTT 6	Uttarakhand	Tomato	_
UTT 4	Uttarakhand	Tomato	_
UTB 1	Uttarakhand	Brinjal	_
ORP 1	Odisha	Potato	_
ORB 3	Odisha	Brinjal	_
HPT 11a	Himachal Pradesh	Tomato	_
WBB 8	West Bengal	Brinjal	_

amplification was observed that indicates the absence of attP region which in turn indicated that the isolated phage is having biocontrol potential.

Evaluation of medium for mass multiplication of actinomycetes

In planta study using nine different actinomycetes revealed the efficacy of IISR Act 4 (*Streptomyces* sp.) in controlling *R. solanacearum*. In order to get a suitable multiplication medium for this isolate, different media such as well decomposed Farm yard manure, coir pith, vermicompost and a mixture of vermicompost and coir pith were tested. The data obtained indicates an increase of 10 fold count in vermicompost. There was no multiplication in other three media tested (Fig. 69).

Isolation of bacteria from apoplastic fluid of ginger

The pseudo stem and leaves were used for the apoplastic fluid extraction using vacuum infiltration and centrifugation method. The apoplastic fluid obtained was serially diluted and plated on tryptic soy agar medium. The distinct bacterial colonies obtained were purified and stored (Fig. 70). Hundred and fifty bacterial isolates were maintained as glycerol stocks. These apoplastic bacteria are being tested for their biocontrol traits both *in vitro* and *in planta*.

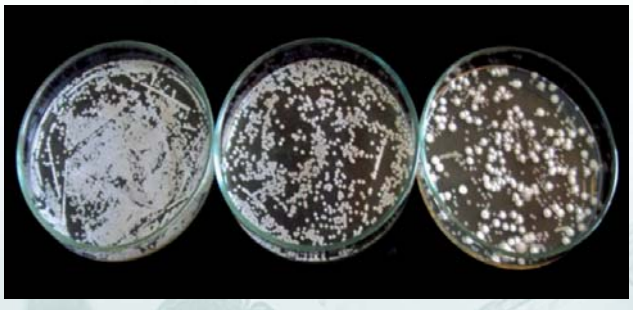


Fig. 69. Multiplication of IISR Act4 in vermicompost

Fig. 70. Inhibition of *R. solanacearum* by apoplastic bacteria

CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA

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Co-Investigators:

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a. *RALSTONIA* DIVERSITY

Collection, isolation and maintenance of *R. solanacearum* isolates

Symptomatic plants/tubers of potato were collected from potato growing areas and a total of 95 isolates of *R. solanacearum* (28 isolates from Bhowali and adjoining areas in Nainital, Uttarakhand and 67 isolates from East Khasi Hill district of Meghalaya) were recovered. Well separated typical wild type *R. solanacearum* were stored in double distilled sterile water at 20±2°C for further studies.

Biovar determination

Strains were classified to biovar using a variation of the physiological test developed by Hayward (1964) which assays ability to oxidize a set of sugars and sugar alcohols. The biovar analysis of isolates revealed that out of 28 isolates collected from Uttarakhand, 12 isolates (42.86%) belonged to biovar 2; whereas 16 isolates (57.14%) belonged to biovar 3. Of the 67 isolates collected from Meghalaya, only 20 isolates (29.9%) belonged to biovar 2. Three isolates (4.5%) were biovar 3; whereas 44 isolates (65.6%) were biovar 2T of the pathogen (Fig.71).

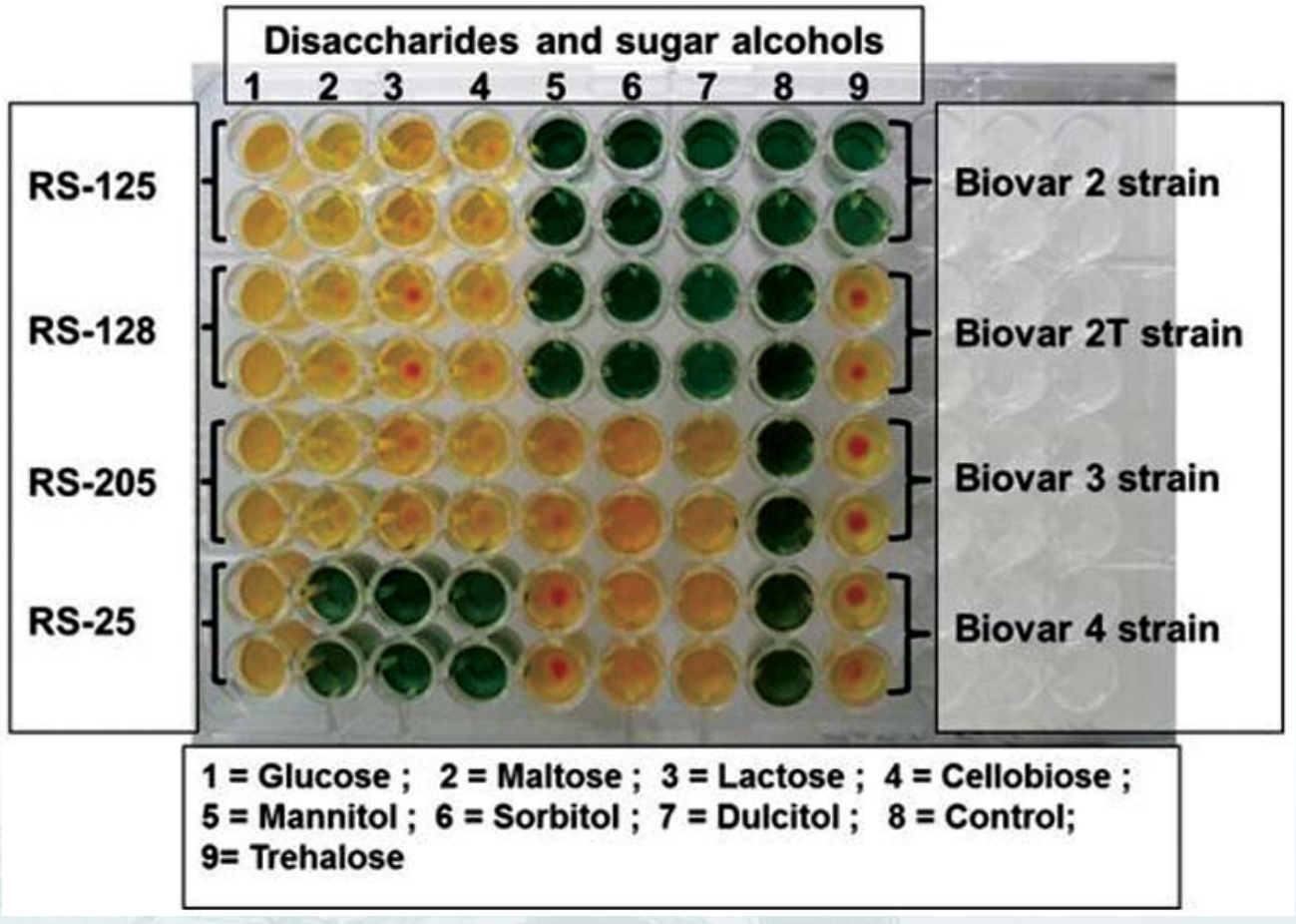

Fig. 71. Micro titer plates showing the results of biovar test of four strains of *R. solanacearum*

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a. *RALSTONIA* DIVERSITY

Bacterial wilt affected tomato and potato plants were collected from Uttarakhand and isolated seven *R. solanacearum* isolates. A total of 159 isolates of *R. solanacearum* from tomato, brinjal, chilli, capsicum and potato were preserved.

Study on genetic divergence among race 1, biovar 3 and 4 of *R. solanacearum* using multi locus sequence typing

Multi locus sequence typing was done for 18 *R. solanacearum* isolates of tomato obtained from different agro-climatic conditions of Jharkhand (JHT-1, JHT-2, JHT-1P, JHT-15), Odisha (ORT-6, ORT-7, ORT-8, ORT-9, ORT-11), Himachal Pradesh (HPT-2, HPT-3, HPT-11 HPT-19b) and Uttarakhand (UTT-11, UTT-22, UTT-23, UTT-24, UTT-26), with internal fragment of two housekeeping genes like *gyr*B (subunit B of DNA gyrase), *acn*B (second aconitase), and one virulence gene as *Hrp* (regulatory transcription regulation).

All the 18 isolates of *R. solanacearum* were amplified for *acn*B at 527 bp (Fig.72). The nucleotide sequences of these isolates along with sequences of *R. solanacearum* isolates of other countries and other related genera such as *Burkholderia* and *Acidovarax* obtained from NCBI database were analyzed. The evolutionary distance was computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The analysis involved total of 26 nucleotide sequences. There were total 314 positions in the final dataset.

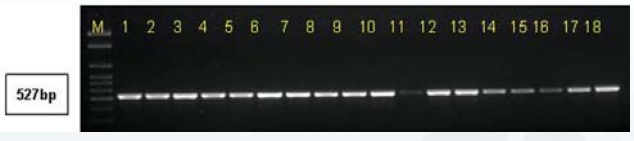

Fig. 72. The amplified DNA fragments of *R. solanacearum* by *acn*B primers. Lane M: 1kb DNA ladder, lanes 1- 4: JHT-1, JHT-2, JHT-1P, JHT-15 (Jharkhand), 5- 9: ORT-6, ORT-7, ORT-8, ORT-9, ORT-11 (Odisha), 10-13: (UTT-11, UTT-22, UTT-23, UTT-24 , UTT-26 (Uttarakhand) and 14–18: HPT-2, HPT-3, HPT-11 HPT-19b (Himachal Pradesh)

Similarly, 14 isolates were amplified for *gyr*B at 732bp (Fig.73). All the isolates of *R. solanacearum* from Northern and Eastern parts of India were grouped into clusters. The isolates were not grouped based on agro-climatic regions of India. Sequence of *gyr*B gene was highly matched only with *R. solanacearum* sequence data obtained from NCBI database. Hence, *gyr*B gene may be used as a marker for detection and diagnosis of *R. solanacearum* from seed, planting material, soil and irrigation water. There were a total of 410 positions in the final dataset.

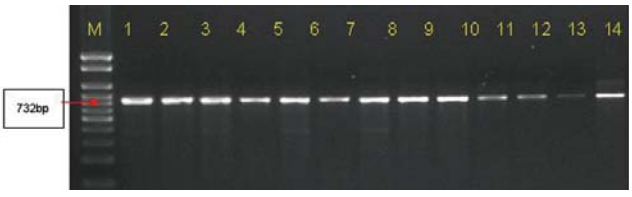

Fig. 73. The amplified DNA fragments of *R. solanacearum* by *gyr*B primers. Lane M: 100 bp DNA ladder, lanes 1- 4: JHT-1, JHT-2, JHT-15 (Jharkhand), 5-9: ORT-6, ORT-7, ORT-8, ORT-9, ORT-11 (Odisha), 10- 13: (UTT-11, UTT-22, UTT-23, UTT-24, UTT-26 (Uttarakhand) and 14 : HPT-3 (Himachal Pradesh)

Twenty five *R. solanacearum* isolates and one isolate of *R. syzygii* R24 was amplified for *hrp*B gene (Fig.74). All 18 isolates from Northern and Eastern parts of India were grouped into three clusters. Isolates UTT-23, UTT-25, JHT-15, HPT-3, HPT-11, UTT-32, ORT-6, HPT-19, UTT-22 and UTT-26 were clustered into one cluster and three Indian isolates *viz.,* ORT-9, JHT-2 and UTT-11 were formed separate groups, whereas, five isolates of *R. solanacearum viz.,* ORT-7, HPT-2, UTT-24, ORT-8 and ORT-11 clustered together. It indicates that the isolates of *R. solanacearum* from Northern and Eastern parts of India have genetic diversity in *hrp*B gene. However all isolates were pathogenic to tomato bacterial wilt.

Phylogenetic analysis

One hundred and ninety sequences were generated from different strains of *R. solanacearum* that includes variety of races and biovars. Each gene was analyzed independently as well as collectively. Only a single copy of each gene studied in this

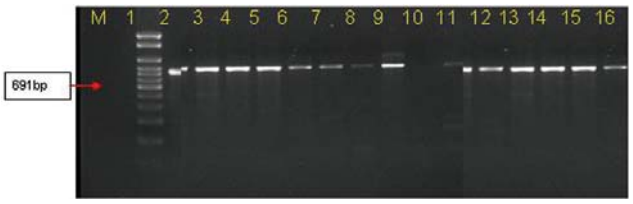

Fig. 74. The amplified DNA fragments by *hrp*B primers for isolates of *R. solanacearum*. Lane name with respective isolates, lane M: 100 bp DNA ladder, lanes 1- 4: JHT-1, JHT-2, JHT-1P, JHT-15 (Jharkhand), 5- 9: ORT-6 , ORT-7, ORT-8, ORT-9, ORT-11 (Odisha), 10- 12: (UTT-11, UTT-22, UTT-23, UTT-24 (Uttarakhand) and 13–16: HPT-2, HPT-11 HPT-19b (Himachal Pradesh)

work was found in the genome of *R. solanacearum* strain GM10000. Phylogenetic tree for each sequence fragment was constructed using the UPGMA, parsimony (MP), Maximum Composite Likelihood method. The Tamura-Nei model with gamma correlations and 1,0000 boot strapping replicates were used to generate UPGMA trees. ML trees were visualized with Tree View that uses series to identify the selective pressures on housekeeping and virulence-related genes of *R. solanacearum*. First, it was determined the d_N/d_S ratios, values of d_N/d_S of 1, d_N/d_S of >1, and d_N/d_S of <1 indicate neutrality, diversifying selection and purifying selection, respectively. *flic* and *egl* showed low level of d_N/d_S (Table 37), indicating that these loci are under strong purifying selection conditions. In contrast, *gdh* and *gyrB* acquired over 1, a d_N/d_S ratio of 1.12 and 1.04 and a d_N/d_S ratio for *acn*B, *hrp*B and adk acquired 2.87, 2.52 and 2.66. This suggested that diversifying selection is determining the evolution of *gdh*, *gyr*B, *acn*B, *hrp*B and *adk* genes. The Tajima's test (D), showed the number of segregating/polymorphic sites and the average number of nucleotide differences (Table 37).

Selection of these genes was based on their use in an MLST scheme of other bacterial species and the availability of some sequence data from the virulence-related *egl* and *hrp*B genes in databases. The virulence-related genes are implicated directly (*egl*) or indirectly (*hrp*B, *fli*C) in disease-causing process. The *egl* gene encodes and endoglucanases that likely act at the front line of host invasion by partially degrading host cell-wall, *hrp*B encodes an *ara*C (1-β-D-arabinofuranosylcystosine) type transcriptional regulatory protein that governs multiple virulence pathways. Flagellin encoded by the *fli*C gene, is the essential subunit of the flagellar filament that is needed for invasion virulence. Fig. 75 reveals that all isolates of *R. solanacearum* isolated from northern and eastern parts of India grouped together except JHT-1 and they were very close to *R. solanacearum* strains CMR15, GRs-Per01, ERs-Cal, GRs-Ktm and Grs-Pkr.

Table 37. Genetic diversity and selection analysis of *R. solanacearum*

Gene	Length (bp)	No. of Sequences	No. of polymorphic sites	% of polymorphic sites	θ[a]	π[b]			Mean Frequency of A\T\G\C	Tajima's (D[c])	$d_N\backslash d_S$
						Total	D_S	D_N			
gyrB	732	26	410	56.0	0.262057	0.688540	7.6	22.9	0.76/0.89/ 1.09/1.27	6.484898	1.12
acnB	527	26	314	59.5	0.262057	0.700392	1.0	9.5	0.90/0.96/. 04/1.10	6.652850	2.87
hrpB	691	26	253	36.6	0.262057	0.514053	4.2	10.5	0.90/0.96/ 1.00/1.10	3.817461	2.52
adk	536	28	332	61.9	0.222175	0.100364	1.0	10.5	0.21/0.51/ 1.23/0.23	-2.155691	2.66
*fli*C	390	28	347	11.23	0.253323	0.188650	4.2	10.5	0.90/0.96/ 1.00/1.10	-1.004125	0.92
egl	850	28	749	11.3	0.255948	0.341421	2.7	8.1	0.90/0.96/ 1.04/1.10	1.318668	0.28
gdh	1000	28	900	90	0.256973	0.657501	7.7	21.4	0.17/0.46/ 0.78/0.35	6.158193	1.04

[a] Theta value per site (Watterson estimator),[b] Nucleotide diversity calculated with Jukes-Cantor correlation,[c] Non significant Tajima D values at a P value of > 0.05, D_S= synonymous, D_N=non- synonymous

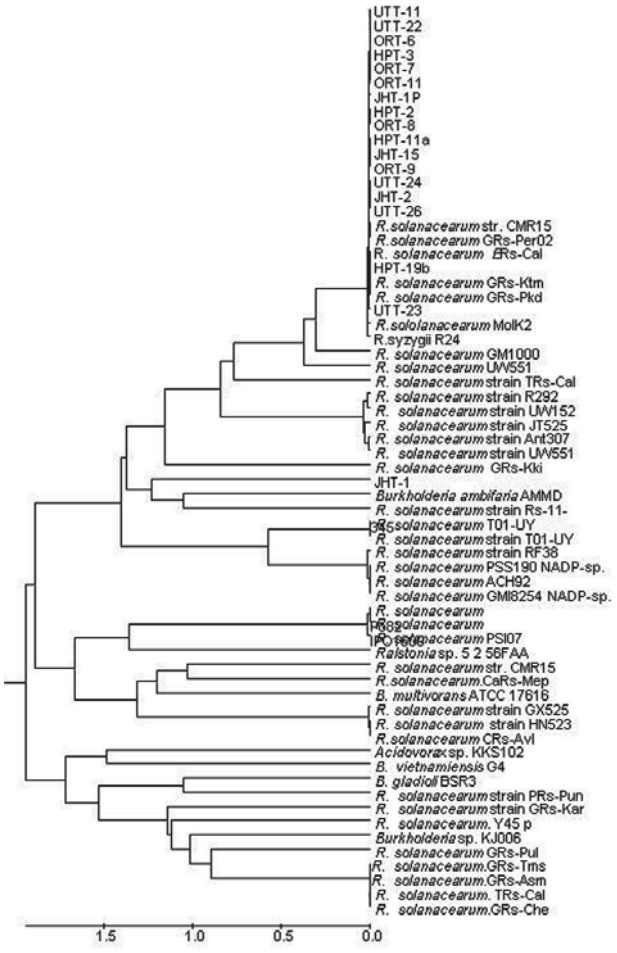

Fig. 75. Phylogenetic analysis of using sequence data of housekeeping genes and virulence genes of *R. solanacearum* and related species, *Burkholderia* spp. and *Acidovarax* spp. The evolutionary history was inferred using the UPGMA method. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The analysis involved 64 nucleotide sequences

b. HOST RESISTANCE

Study on the effect of jasmonic acid, salicylic acid and antagonistic bacteria on expression of defense related gene (S) in tomato cultivars against *R. solanacearum*

Expression of defense related genes *viz*., *PR*1a and *Glu*A (salicylic acid pathway), *Pin*2 and *Lox*A (Jasmonic acid pathway) and *PR*1b and Osmotin like (ethylene pathway) in resistant (Hawaii 9796) and susceptible (Pusa Ruby) tomato cultivars against the *R. solanacearum* strain UTT-24 were assessed after treatments with chemical inducers of defense like jasmonic acid and salicylic acid with and without *Bacillus subtilis* (Table 38). The experiment was conducted at Phytotron in growth chamber. The samples were collected at different duration (6, 24, and 48, 96 h and 60 days). The amplified product of *PR*1a in Pusa Ruby and Hawaii 9796 was given in Fig. 76 and 77. In this study, gene expression was quantified separately for each cDNA samples. Genes represent activation of the (*PIN*2, *Lox*A), the Ethylene pathway (*PR*1b, Osmotin like) and salicylic pathway (*Glu*, *PR*1a) were semi-quantitatively analyzed by agarose-gel electrophoresis in response to pathogen cell densities. Amplification with *PIN*2, *Lox*A showed negative in all the treated cDNA samples. Among *PR*1b and osmotin like genes, only osmotin was amplified in all the samples. *Glu*, *PR*1a genes showed amplification in all the treated cDNA samples. The expression profile of *PR*1a was highly variable irrespective of the treatments and may not reflect the accurate gene activity. In comparison, the expression level was high in susceptible cultivar if the genes analyzed separately.

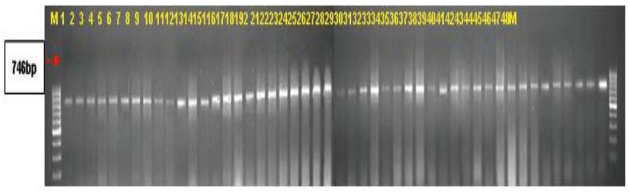

Fig. 76. Semi-quantitative analysis of Pusa Ruby (susceptible) with PR-1a primer at 746 bp. Lane M= 100bp, Lanes 1-4 (PR+UN), 5-8 (PR+RS), 9-12 (PR+BS), 13-16 (PR+RS+BS), 17-20 (PR+JA), 21-24 (PR+JA+RS), 25-28 (PR+JA+BS), 29-32 (PR+JA+RS+BS), 33-36 (PR+SA), 37-40 (PR+SA+RS), 41-44 (PR+SA+BS), 45-48 (PR+SA+RS+BS), 6, 24, 48 and 60 days treated cDNA samples

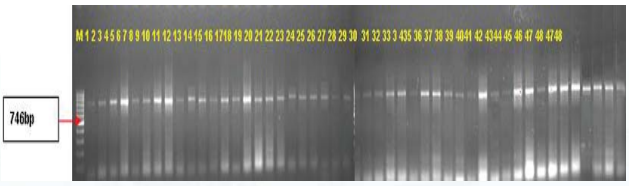

Fig. 77. Semi-quantitative analysis of Hawaii-9796 (resistant) with PR-1a primer at 746 bp. Lane M= 100bp, Lanes 1-4 (HW+UN), 5-8 (HW+RS), 9-12 (HW+BS), 13-16 (HW+RS+BS), 17-20 (HW+JA), 21-24 (HW+JA+RS), 25-28 (HW+JA+BS), 29-32 (HW+JA+RS+BS), 33-36 (HW+SA), 37-40 (HW+SA+RS), 41-44 (HW+SA+BS), 45-48 (HW+SA+RS+BS) 6, 24, 48 and 60 days treated cDNA samples

Effect of antagonistic bacteria on induction of defense enzymes in tomato against *R. solanacearum*

Experiments were conducted at Phytotron, IARI, New Delhi to study the induction of five induced systemic resistant enzymes. Two tomato cultivars, *viz.,* Arka Abha (resistant) and Pusa Ruby (susceptible) to tomato bacterial wilt were studied. Seeds of both the cultivars were treated with *B. subtilis, R. solanacearum*, and untreated seeds kept as control. The leaf samples were collected for enzyme extraction at 6, 24, 48, 72 and 96h after inoculation. Induced systemic resistance (ISR) enzyme *viz.,* super oxide dismutase (SOD), peroxidase, polyphenol oxidase, catalase, and phenylalanine ammonia–lyase (PAL) were assayed as per standard procedure (Soares *et al.* 2005). Minimum bacterial wilt disease intensity (15.0 % ± 2.31) was recorded in Arka Abha treated with *B. subtilis* after 60 days of inoculation (Table 38). The root and shoot length of resistant (Arka Abha) and susceptible (Pusa Ruby) cultivars were slightly lower in *R. solanacearum* inoculated plants than uninoculated and treated with antagonistic bacteria. The root and shoot weight of resistant (Arka Abha) and susceptible (Pusa Ruby) cultivars were slightly lower in *R. solanacearum* inoculated plants than uninoculated and treated with antagonistic bacteria. Whereas, the root and shoot dry weight of resistant (Arka Abha) and susceptible (Pusa Ruby) cultivars were slightly lower in *R. solanacearum* inoculated plants than uninoculated and treated with *B. subtilis.*

The maximum activity of PAL, PPO, POD, SOD and Catalase occurred in different duration in plants (Table 39). The overall results showed that *B. subtilis* treatments significantly increased the activity in resistant cultivar (Arka Abha), were as susceptible cultivar (Pusa Ruby) inoculated with *R. solanacearum* showed significantly highest enzyme activity than *B. subtilis* at 96h after inoculation.

c. DISEASE MANAGEMENT

Screening of tomato cultivars against bacterial wilt under field conditions

Nine tomato cultivars were evaluated for bacterial wilt at farmer's field at Chorgaliya village, Nanital district, Uttarakhand. Minimum disease incidence (1.2%) was recorded in Hawaii 9796 followed by Arka Rakshak (4.0%) and Arka Samarat (4.5%). Number of plant was higher in Pusa Ruby (86.3 cm) followed by Arka Samrat (79.2 cm) whereas,

Table 38. Disease incidence and growth parameters of susceptible and resistant cultivars treated with antagonistic *B. subtilis* against *R. solanacearum*

Treatment	Plant height (cm)		Plant fresh weight (g)		Plant dry weight (g)		Bacterial wilt incidence (%)
	Root	Shoot	Root	Shoot	Root	Shoot	
Pusa Ruby - uninoculated	5.0 ± 1.5	56.0 ± 12.1	9.8 ± 1.3	186.2 ± 18.4	1.8 ± 0.4	14.1± 0.2	0.0
Pusa Ruby + *R. solanacearum*	4.8 ± 3.1	50.3 ± 17.7	8.8 ± 2.5	106.1 ± 65.3	1.6 ± 0.3	11.9 ± 1.2	80.0 ± 4.0
Pusa Ruby + *B. subtilis*	5.6 ± 0.2	62.5 ± 8.5	11.0 ± 1.6	189.5± 51.1	2.1 ± 0.6	14.4 ± 0.8	0.0
Pusa Ruby + *B. subtilis* + *R. solanacearum*	5.7 ± 2.2	60.7 ± 14.4	9.2 ± 2.0	181.7 ± 50.8	1.6 ± 0.3	12.9 ± 1.2	45.67 ± 4.6
Arka Abha – uninoculated	3.9 ± 1.0	57.0 ± 14.6	12.7 ± 3.2	175.9 ± 38.2	2.1 ± 0.6	13.8 ± 2.0	0.0
Arka Abha + *R. solanacearum*	3.7 ± 0.5	50.9 ± 14.2	9.5 ± 2.3	115.9 ± 54.8	1.8 ± 0.3	12.2 ± 1.6	25.0 ± 4.3
Arka Abha + *B. subtilis*	4.6 ± 0.7	56.4 ± 8.9	13.6 ± 4.5	187.6 ± 96.5	2.2 ± 0.6	13.9 ± 1.6	0.0
Arka Abha + *B. subtilis* + *R. solanacearum*	4.9 ± 1.2	53.4 ± 6.6	12.5 ± 2.6	164.5 ± 31.6	1.7 ± 0.2	12.5 ± 0.3	15.0 ± 2.31

Table 39. Induced systemic resistance enzymes produced by resistant and susceptible cultivars of tomato treated with antagonistic *B. subtilis* against *R. solanacearum*

Treatment	Enzymatic activity									
	POD (specific activity / min/ mg protein)		PPO (specific activity / min/ mg protein)		SOD (specific activity / U/ g)		PAL (specific activity / U/ g)		Catalase (specific activity / min/ mg protein)	
	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
Pusa Ruby-uninoculated	0.076	0.04	0.076	0.16	98.5	98.5	574.0	616.6	5.7	5.74
Pusa Ruby + *R. solanacearum*	0.002	0.05	0.002	0.074	98.4	97.8	671.4	671.7	5.8	5.66
Pusa Ruby + *B. subtilis*	0.088	0.08	0.09	0.11	98.4	98.7	590.3	621.7	5.7	5.42
Pusa Ruby + *B. subtilis* + *R. solanacearum*	0.078	0.044	0.078	0.07	98.5	98.4	611.4	620.0	5.6	5.78
Arka Abha – uninoculated	0.05	0.06	0.057	0.08	99.1	98.5	802.0	890.0	5.7	5.83
Arka Abha + *R. solanacearum*	0.07	0.07	0.07	0.08	99.9	97.2	844.0	853.4	5.8	5.87
Arka Abha + *B. subtilis*	0.08	0.099	0.077	0.08	98.6	98.3	849.7	863.0	5.9	5.86
Arka Abha + *B. subtilis* + *R. solanacearum*	0.06	0.02	0.06	0.08	99.0	98.5	816.0	821.4	5.9	5.44

PPO = peroxidase, POD = polyphenol oxidase, SOD = Superoxide dismutase, PAL = phenylalanine ammonia.

fruits weight was higher in Arka Samarat (81.09 g) followed by Hybrid Sivam (69.0 g). Maximum number of fruist were also higher in Arka Samrat (69 fruits) followed by Pusa Ruby (44 fruits) (Table 40). However, Hawaii 9796 showed resistant against bacterial wilt but its fruit quality and fruiting was lower than Arka Samarat. Arka Samrat may be included in integrated bacterial wilt disease management to reduce disease incidence and reduce the crop loss.

Integrated management of bacterial wilt under field conditions

To manage tomato bacterial wilt, an experiment

Table 40. Evaluation of tomato cultivars against bacterial wilt at farmer's field

Cultivar	Bacterial wilt (%)	Plant height (cm)	Fruits/plant	Fruit weight (g)
Arka Samarat (resistant)	4.5	79.2	69	81.0
Pusa Ruby (susceptible)	17.5	86.3	44	55.0
Hawaii 9796 (resistant)	1.2	70.5	41	47.0
Arka Abha (resistant)	6.0	69.5	32	57.5
Pusa Sheetal (moderate)	9.0	69.5	24	45.0
Arka Rakshak (resistant)	4.0	64.7	26	56.5
Pusa Sadabahar (moderate)	10.5	63.9	30	49.5
P-120 (moderate)	10.0	50.2	39	48.0
Hybrid Shivam (control)	11.0	57.6	42	69.0

on integrated disease management was conducted at farmer's field at chorgaliya, Nanital, Uttarkhand. The bioagents *viz*., *Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis* alone or combination with bleaching powder was applied at root zone of each plant during transplanting. Bleaching powder @15 kg/ ha was applied in ridges and furrows. Two cultivars of tomato *viz*., resistant (Arka Samrat) and susceptible (Pusa Ruby) were transplanted in each treatment. No disease was recorded in biocontrol agent in combination with bleaching powder in both the cultivars. However, susceptible cultivar (Pusa Ruby) showed higher disease incidence as compared to resistant (Arka Abha) cultivar in all the treatments (Table 41). *P. fluorescens* reduced disease incidence better than other bioagents and significant variation was found within the treatments.

Detection of antibiotic producing antagonistic *Bacillus* species through PCR

Protocol for identification of antibiotic genes *viz*., Subtilin, Bacilysin A and Bacilysin B of *Bacillus* spp. was standardized. The amplification of subtilin gene was obtained at 709 bp while, other two genes, Bacilysin A and Bacilysin B was at 503bp and 512 bp, respectively. Out of 47 isolates of *Bacillus* spp. including *B. cereus, B. licheniformis* and *B. subtilis*, only six isolates *viz.,* DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, UTTBS-1 and *B. subtilis*, showed positive amplification of Subtilin gene at 709 bp, while Bacilysin A gene amplified at 503bp showed positive by 12 isolates *viz*., METBE-35, DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, JHTBS-5, UTTBS-1,METBS-32, DTBS-5, JHTBS-8, *B. subtilis*. *B. licheniformis* and Bacilysin B gene was amplified at 512 bp produce only nine strains METBE-35, DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, JHTBS-5, UTTBS-1, METBS-32, *B. subtilis*. Moreover, *B. subtilis*, *Bacillus* spp. isolates DTBSE-4, UTBS-1, UTTBS-3, JHTBS-2 and HPTBS-4 showed amplification in all the three antibiotic producing genes *viz*., Subtilin, Bacilysin (Bac A) and Bacilysin (Bac B) (Table 42).

Table 41. Integrated disease management of tomato bacterial wilt at field level

Treatment	Cultivar					
	Arka Samrat			Pusa Ruby		
	Bacterial wilt (%)	Plant height (cm)	Fruits/ plant	Bacterial wilt (%)	Plant height (cm)	Fruits / plant
T. harzianum Th3	4.5	65.1	84	9.0	82.7	110
P. fluorescens, pf5	3.5	84.9	76	6.5	84.1	33
B. subtilis DTBS -5	4.5	85.4	119	8.0	71.6	36
P. fluorescens + *B. subtilis*	0	83.7	68	0	88.5	49
T. harzianum + bleaching powder	0	78.9	35	0	90.1	31
P. fluorescens + bleaching powder	0	99.3	85	0	90.1	33
B. subtilis + bleaching powder	0	75.9	141	0	96.9	48
P. fluorescens + *B. subtilis* + bleaching powder	0	85.8	123	0	93.4	23
Control	70	72.2	56	17.5	78.2	40

Table 42. Screening of antibiotic producing antagonistic *Bacillus* spp. isolated from rhizospheric soil and endophytically tomato plants

Name of the antibiotic	Antibiotic positive	Antibiotic negative
Bacilysin (Bac A)	METBE-35, DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, JHTBS-5, UTTBS -1, METBS-32, DTBS-5, JHTBS-8., *B. subtilis*, *B. licheniformis*	MTBS-1, MTBS-2, MTBE-1, MTBE-2, MTBS-3, MTBS-4, MTBE-3, MTBE-4, MTBS-5, MT*BS*-6, MT*BE-5,* MTBS-6, MTBE-5, JHTBS-6, JHTBS-7, JHTBS-9, DTBS-6, DTBE-8, DTBE-9, JTBS-9, JTBE-14, JTBS-17, JTBE-20, JTBS-21, JTBE-23, UTBS-24, UTBE-25, UTBE-26, UTBS-32, UTBS-33, UTBE-29, UTBS-30, UTBE-30, UTBE-31, METBS-31, *B. cereus*
Bacilysin (Bac B)	METBE-35, DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, JHTBS-5, UTTBS-1, METBS-32, *B. subtilis*	MTBS-1, MTBS-2 MTBE-1, MTBE-2, MTBS-3, MTBS-4, MTBE-3, MTBE-4, MTBS-5, MT*BS*-6, MT*BE-5,* MTBS-6, MTBE-5, JHTBS-6, JHTBS-7, JHTBS-8 , JHTBS-9, DTBS-5, DTBS-6, DTBE-8, DTBE-9, JTBS-9, JTBE-14, JTBS-17, JTBE-20, JTBS-21, JTBE-23, UTBS-24, UTBE-25, UTBE-26, UTBS-32, UTBS-33, UTBE-29, UTBS-30, UTBE-30, UTBE-31, METBS-31, *B. cereus*
Subtilin	DTBSE-4, JHTBS-2, UTTBS-3, HPTBS-4, UTTBS-1, *B. subtilis*	MTBS-1, MTBS-2 MTBE-1, MTBE-2, MTBS-3, MTBS-4, MTBE-3, MTBE-4, MTBS-5, MT*BS*-6, MT*BE-5,* MTBS-6, MTBE-5, JHTBS-6, JHTBS-7, JHTBS-7, JHTBS-8 , JHTBS-9, DTBS-5, DTBS-6, DTBE-8, DTBE-9, JTBS-9, JTBE-14, JTBS-17, JTBE-20, JTBS-21, JTBE-23, UTBS-24, UTBE-25, UTBE-26, UTBS-32, UTBS-33, UTBE-29, UTBS-30, UTBE-30, UTBE-31, METBS-31, *B. cereus.* *B. licheniformis*

ICAR RESEARCH COMPLEX FOR GOA, GOA

Principal Investigator:
Dr. R. Ramesh

Co-Investigator:
Dr. M. Thangam

a. *RALSTONIA* DIVERSITY

R. solanacearum isolates (232) collected from different crops *viz*., brinjal, chilli and tomato from Goa (74 different places, 149 isolates); Karnataka (15 different places, 18 isolates), Kerala (11 different places, 20 isolates), Maharashtra (15 different places, 19 isolates) and Andaman Islands (25 places, 25 isolates) are preserved in 30 % glycerol at -70 °C (Fig. 78).

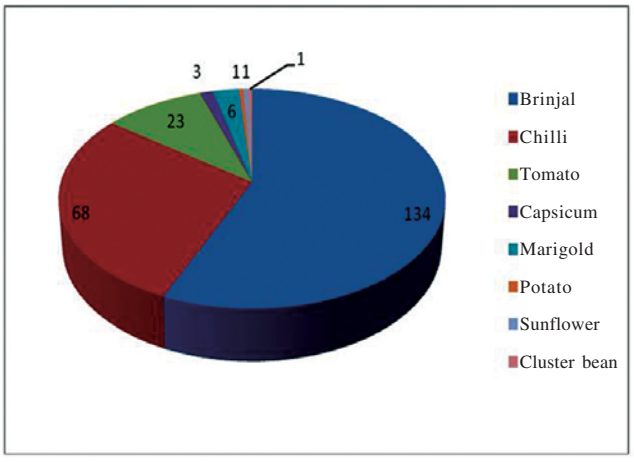

Fig. 78. *Ralstonia* collections (cropwise)

Virulence of *R. solanacearum* isolates

Virulence of the isolates (196) collected from different regions was tested on brinjal seedlings. The disease incidence was recorded as percent disease index from seven days after inoculation. Results indicated that the isolates vary in virulence on brinjal.

Multi Locus Sequence Typing (MLST)

Twenty diverse strains were selected based on the *egl* sequence tree, geographical location and host for MLST analysis. Five chromosomal housekeeping genes namely *gapA*, *ppsA*, *gdhA*, adk and *gyrB* and one megaplasmid based virulence gene *fliC* was included in the study along with *hrp*B and *egl*. All the gene products were sequenced, the sequences were submitted to NCBI and accession numbers were obtained. Analysis of sequence data was carried out by two different methods. The MLST scheme suggested by Castillo and Greenberg (2007) was adopted in the first case. The sequences of the isolates were compared in the MLST database and the allele numbers were assigned. Results indicated that some of the isolates are assigned same allelic profile (Rs-08-55, Rs-10-204, Rs-10-292, Rs-09-193; Rs-10-253, Rs-10-257; Rs10-336, Rs-09-131). Another approach to analyze the data based on a worldwide collection of plant pathogenic *R. solanacearum* database. After alignment with 60 reference *egl* sequences, strains are assigned to nine *egl* haplotypes, among which five are new. From the six gene concatenate, 15 haplotypes are found within Indian strains. All of which are new, different from the 88 reference haplotypes and of the 21 phylotype I haplotypes. Statistical analyses indicated that 1) Indian strains are related to each other and that recombination occurred across these strains 2) Significant recombination among Indian strains and total phylotype I strains 3) Recombination/ mutation ratio is low, suggesting that sequences evolve more by mutation than recombination and 4) Indian strains look clearly original, and deserve further investigations

b. HOST RESISTANCE

Crosses were made between Surya and Agassaim to develop F_1 and F_2 populations. Seedlings from F_2 cross seeds were raised along with the resistant and susceptible parent lines for collection of F_3 seeds and for further evaluation. Results indicated that all the plants of Agassaim wilted but none of the plants of resistant donor. In F_2 population some plants did not wilt. Seeds were collected from the F_2 plants which are not wilted to test their resistance in the next generation and for further molecular analysis.

c. DISEASE MANAGEMENT

Exploration of xylem residing microbes

Twenty eight xylem residing bacteria (XRB) with antagonistic activities were screened for the production antimicrobial products, plant growth promoting substances and exoenzymes. 17.85% isolates produced HCN. All the fluorescent isolates produced iron scavenging siderophores and

31.14% isolates produced esterases which have a potential to be used as antagonists against *R. solanacearum*. Majority of the isolates produced indole acetic acid with concentrations ranging from 15 µg/ml to 615 µg/ml. 64.28 % of the isolates solubilized phosphate. 25% of the isolates produced cellulase and amylase where as 14.28 % isolates produced pectinases. 32.14% isolates produced protease.

Xylem bacteria were screened for the production of antagonistic compounds against *R. solanacearum* by standard bioassay. Isolates were tested against mildly virulent, moderately virulent and highly virulent strains of *R. solanacearum*. The inhibition zone ranged from 11-61mm. 28 antagonistic isolates was evaluated for growth promotion in brinjal under greenhouse conditions. Based on the observations six xylem residing bacteria namely XB100, XB122, XB1, XB27, XB202 and XB203 increased the growth in brinjal as compared to the un-inoculated control (Fig. 79).

Plant products for bacterial wilt suppression

The crude extract prepared with different organic solvents from local and wild plant species was used in the bioassay against the pathogen. Results indicated that some of the plant products exhibit inhibition of *R. solanacearum* when tested *in vitro*.

Fig. 79. Plant growth promotion in brinjal by XRB

INDIAN INSTITUTE OF HORTICULTURAL RESEARCH, BENGALURU

Principal Investigator:
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Co-Investigator:
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a. *RALSTONIA* DIVERSITY

Twenty *R. solanacearum* isolates were collected from wilted tomato, pepper and eggplant at different locations. The 12 representative isolates were used for further analysis using *R. solanacearum* specific primers for confirmation as *R. solanacearum*. The isolates were identified by specific amplification of 370bp DNA fragment. The confirmed samples were used for further characterization.

b. DIAGNOSTICS

PCR amplification of 16S rDNA gene

The PCR amplification of DNA from the isolates using MKRSF1/MKRSR1 primers yielded expected 1.5 kb fragment. Sequencing of 16S rRNA gene of six isolates indicated that the gene has 1462 nucleotides. One brinjal (BRS57) and four tomato isolates (TRS22, 24, 25, and 26) have 90.5 to 96.7% homology among six isolates. Phylogenetic analysis of 16S rDNA gene sequences showed clustering of TRS22, 24, 25 and 26 isolates along with one Indian (JQ217369) and Chinese isolates (JF700383, FJ210682, FJ210681). The brinjal isolate BRS57 clustered as separate sub group along with Chinese isolate (FJ184057). The tomato isolate TRS27 formed another clade along with Chinese isolate (HQ176322) (Fig. 80).

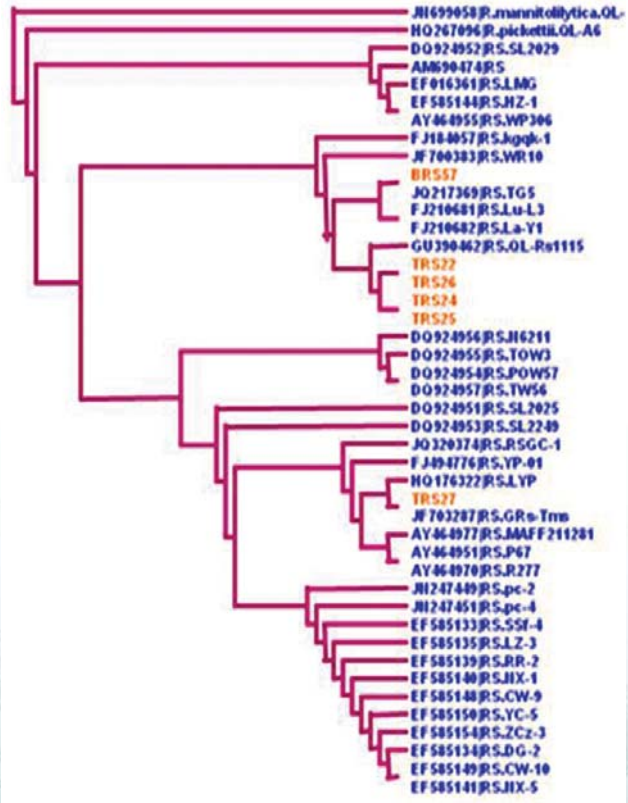

Fig. 80. Phylogenetic analysis of 16s rDNA gene

c. EPIDEMIOLOGY

Interaction between nematode (*Meloidogyne incognita*) and *R. solanacearum* in disease development

The experiment on interaction of nematode and bacterium in the development of wilt disease in tomato was carried out under glasshouse condition with various treatments and combinations. Results indicated that *R. solanacearum* (RS) alone caused 80 to 100 % wilting of tomato when the bacterial concentration ranged from 10^6 to 10^{10} cfu/kg soil. Whereas, *M. incognita* (MI) caused 0 to 85 % wilting at the concentration of 10^1 to 10^5 juveniles/ kg soil. Complete wilting was recorded in the combined treatment of RS at 10^{10} and 10^9 cfu/ kg soil and MI at 10^1 to 10^5 juveniles/ kg soil. MI at 10^4 and 10^5 juveniles/kg soil along with RS at 10^6 to 10^8cfu/ml caused 90 to 100 % wilting indicating clear interaction between the nematode and the bacterium. It is confirmed that the nematode population of 10^3 juveniles along with bacterial population of 10^9 cfu per kg of soil can trigger the wilting symptom in tomato.

d. GENOMICS

PCR Amplification of regulatory genes such as *hrp*B gene, glutamate dehydrogenase oxidoreductase (*gdh*A) gene and endoglu-canase gene

The oligonucleotide primers for amplification of regulatory genes such as *hrp*B and endoglucase genes of *R. solanacearum* were designed based on the sequence of operon available in the NCBI data base. Accordingly, primer pair RSHRPBF/ RSHRPBR, GDHAF/GDHAR and EglF/EglR was designed. The *hrp, gdh* and endoglucanase genes of the 18 isolates were amplified. All the amplified

DNA fragments were gel purified, cloned and sequenced. Sequence analysis of *hrp* gene segment was employed to characterize *Ralstonia* isolates obtained from wilted tomato, Chilli, and eggplant from different locations of Karnataka, Kerala, Delhi and Goa. The comparative sequence analysis revealed that the isolates from India have difference with respect to already reported isolates of *R. solanacearum* and other *Ralstonia* species. Isolates from Karnataka were closely related among themselves (identity range between 95.8 to 99.8%). Isolates from Karnataka showed 92.5 to 94.5% identity with isolates from Goa and these showed 95.7 to 99.7% identity with isolates from Kerala. Indian isolates showed 73.1 to 81.3% identity to isolate from Japan (*R. solanacearum* strain: MAFF 211493).

Of the 18 isolates, 12 isolates amplified a fragment of 600bp and four isolates amplified a fragment of 900bp for GDHAF/GDHAR primer. The 12 isolates that amplified a fragment of 600bp gave non specific sequences whereas the four isolates which amplified a fragment of 900bp gave 99% identity to glutamate dehydrogenase oxidoreductase (*gdh*A) gene (AL646052). Of the 18 isolates, only six isolates amplified a fragment of 0.9kb for endoglucanase gene; where as in control and 12 isolates did not amplify any band of expected size.

Genomic fingerprinting of *Ralstonia* isolates using repetitive sequence based polymerase chain reaction

This genomic fingerprinting method was employed based on DNA primers corresponding to naturally occurring interspersed repetitive elements in bacteria, such as the ERIC elements, and the PCR reaction (rep-PCR). It has been shown that ERIC-PCR fingerprinting is a highly reproducible and simple method to distinguish closely related strains, to deduce phylogenetic relationships between strains and to study their diversity in a variety of ecosystems. Computer assisted pattern analysis programs can be used for microbial identification and phylogenetic analysis of complex data sets, and for the creation of databases for bacterial diagnosis (Fig. 81). Using NTSYSpc program the phylogenetic tree was obtained.

e. HOST RESISTANCE

Standardization of inoculation methods to screen tomato, brinjal and chilli entries for bacterial wilt resistance

Three methods of inoculations *viz*., soil drenching, leaf clipping and axil puncturing were tested for screening tomato, brinjal and chilli entries for

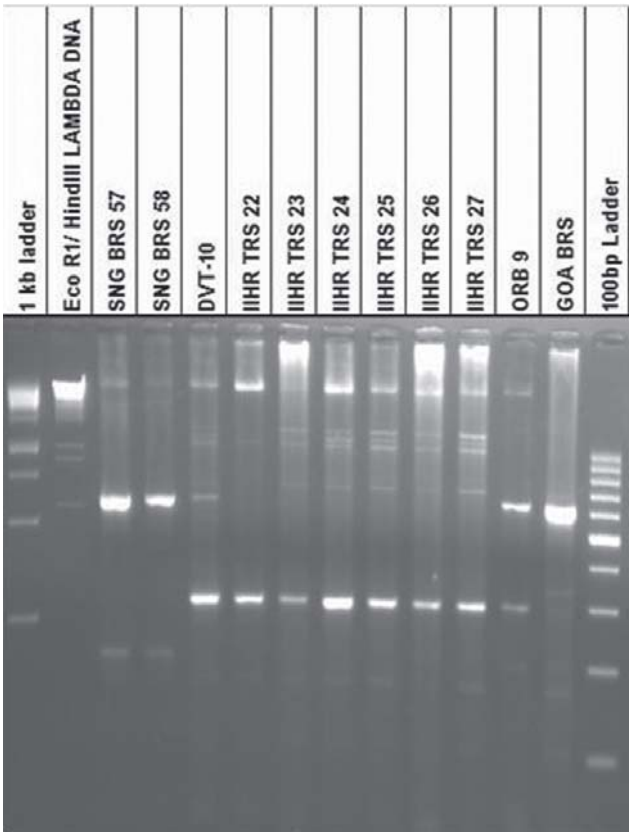

Fig. 81. Genomic fingerprinting of *Ralstonia* isolates by ERIC PCR

bacterial wilt resistance (Fig. 82). The experiment was carried out under glasshouse condition. Results showed that the soil drenching method of inoculation was the best in all the three crops tested, as compared to leaf clipping and axil puncturing methods. The inoculation through soil drenching recorded significantly highest bacterial wilt incidence of 98, 95 and 90 % in tomato, brinjal and chilli, respectively, followed by inoculation through axil puncturing which recorded 78, 88 and 78 % wilt incidence (Table 43). In case of tomato, the leaf clipping method, however, recorded 74 % wilt incidence, which was statistically on par with wilt incidence recorded in axil puncturing method. Whereas, in case of brinjal and chilli, the leaf clipping method of inoculation recorded very low per cent wilting of 48 and 40 respectively, and appears to be less effective. In soil inoculation method the disease symptom was first noticed on 5th day of inoculation in tomato and on 7th day of inoculation in brinjal and chilli. In case of axil puncturing the disease symptom appeared on 7thday of inoculation in all the three crops, whereas in leaf clipping method the disease symptom appeared on 9th day of inoculation in brinjal and chilli. The incubation period in tomato ranged from

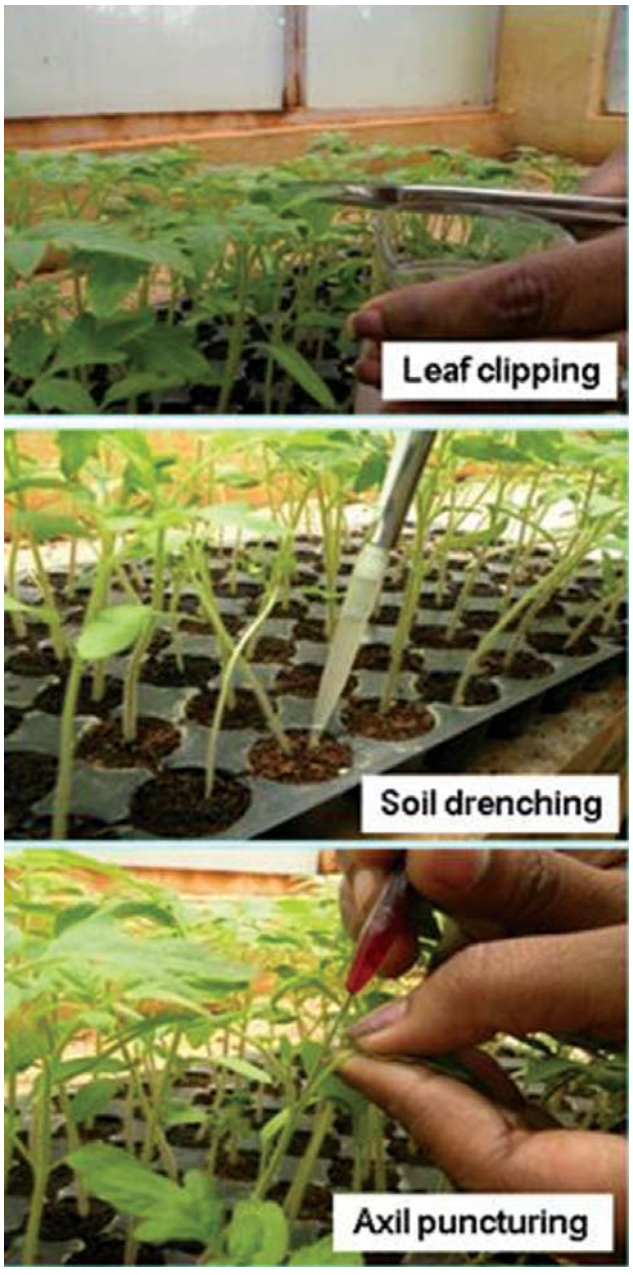

Fig. 82. Standardization of different inoculation methods on bacterial wilt incidence

5 to 21 days, whereas in brinjal and chilli it was 7 - 30 days, this may be due to the hardiness of the plants.

f. DISEASE MANAGEMENT

Testing field bio-efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against bacterial wilt in tomato

The efficacy of the potential bioagents, *P. fluorescens* (Pf) and *B. subtilis* (Bs) against bacterial wilt in tomato variety Shivam (bacterial wilt susceptible) was tested under bacterial wilt sick plot. Results showed that the bacterial wilt incidence was significantly low (9%) with highest yield of 28.8 t/ha in seed treatment followed by soil drenching with Pf was imposed as compared to highest wilt incidence of 62% with lowest yield of 8.25 t/ha in untreated control (Fig. 83, Table 44). The next best treatment was seed treatment plus soil drenching with Bs, which recorded wilt incidence of 12.5% and yield of 25.4 t/ha, respectively.

Field evaluation of chemicals, FYM, green manure and bioagents against bacterial wilt in tomato

A field trial was carried out for the integrated management of bacterial wilt in tomato variety Shivam (susceptible) using chemicals, FYM, green manure and bioagents. Results indicated that the wilt incidence was lowest (10.20%) with increased yield of 39.60 t/ha in combined treatment of FYM + GM + Pf + COC (0.2%). This was followed by the treatments, FYM + GM + Bs + COC, *P. fluorescens* and *B. subtilis* alone. Thus, seed treatment with *P. fluorescens* before sowing and transplanting in soil amended with FYM and green manure gave good control of bacterial wilt in tomato (Table 45).

Table 43. Effect of different inoculation methods on per cent bacterial wilt incidence in tomato, brinjal and chilli

Method of inoculation	Tomato (Arka Vikas)		Brinjal (Arka Shirish)		Chilli (PBC 1367)	
	BW (%)*	Incubation (days)	BW (%)*	Incubation (days)	BW (%)*	Incubation (days)
Soil drenching	98.00 (84.29)	5 - 21	95.00 (77.42)	7 - 30	90.00 (71.74)	7 - 30
Leaf clipping	74.00 (59.34)	7 - 21	48.00 (43.84)	9 - 30	40.00 (39.20)	9 - 30
Axil puncturing	78.00 (62.04)	7 - 21	88.00 (70.03)	7 - 30	78.00 (62.08)	7 - 30
CD (P=0.05)	4.08		3.55		4.08	

*Mean of eight replications; figures in parentheses are arc sine transformed values

Table 44. Field bio-efficacy of *P. fluorescens* and *B. subtilis* against bacterial wilt in tomato

Treatment	Mean wilt (%)*	Mean yield (t/ha)*
P. fluorescens - seed treatment	10.0 (18.40)	21.5
P. fluorescens - seedling root dip	14.4 (22.28)	19.8
P. fluorescens - seed treatment + soil drenching	9.0 (17.41)	28.8
B. subtilis - seed treatment	12.0 (20.22)	20.0
B. subtilis - seedling root dip	16.5 (23.93)	16.8
B. subtilis - seed treatment + soil drenching	12.5 (20.65)	25.4
Streptocycline	25.0 (30.00)	14.2
Untreated control	62.0 (51.93)	8.25
CD (P=0.05)	1.89	3.82

*Mean of four replications figures in parenthesis are arc sine transformed values

Fig. 83. Management of bacterial wilt in tomato

Table 45. Integrated management of bacterial wilt in tomato using bioagents, botanicals and bactericide

Treatment	Mean wilt (%)*	Yield (t/ha)*
FYM @ 20 t/ha	22.84 (28.45) ^{bc}	14.60 ^{b}
FYM + green manure (GM) (sunnhemp) @ 25 t/ha	20.00 (26.50) ^{b}	18.92 ^{b}
GM @ 25 t/ha	20.40 (26.77) ^{b}	18.00 ^{b}
P. fluorescens (Pf) @ 10^{8}cfu/ml (seed treatment and Soil drenching)	12.00 (20.06) ^{a}	36.00 ^{a}
B. subtilis (Bs) @ 10^{8}cfu/ml (seed treatment and soil drenching)	12.50 (20.64) ^{a}	35.78 ^{a}
Neem cake @ 150 kg/ha	23.25 (28.76) ^{bc}	18.80 ^{b}
Pongamia cake @ 150 kg/ha	25.68 (30.40) ^{bc}	15.60 ^{b}
FYM + GM + Pf (seed treatment and soil drenching + COC)	10.20 (18.52) ^{a}	39.60 ^{a}
FYM + GM + Bs (seed treatment and soil drenching + COC)	10.90 (19.06) ^{a}	37.82 ^{a}
FYM + GM + Pf + Bs	12.00 (20.00) ^{a}	35.20 ^{a}
Streptocycline 250 ppm + COC 0.2 %	20.00 (26.39) ^{b}	18.90 ^{b}
COC 0.2%	20.48 (26,81) ^{b}	17.90 ^{b}
Untreated control	65.00 (53.72) ^{d}	9.50 ^{c}
CD (P=0.05)	4.29	4.48

*Mean of four replications, figures in parenthesis are arc sine transformed values

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT INSECTS, BENGALURU

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Dr. R. Rangeshwaran (from 12 Dec 12)

Co-Investigators:

Dr. R. Rangeshwaran (upto 11 Dec 12)
Dr. B. Ramanujam

a. BIOLOGICAL CONTROL

Isolation and evaluation of bacteriophages

New isolates of *R. solanacearum* and phages were obtained from new soil samples. The pathogenicity was confirmed by inoculating on brinjal seedlings in net house. The double layer agar method of isolating phages was carried on using *Ralstonia* culture and bacteriophage filtrates in order to the plaque formation. Cross infectivity of different phages and *Ralstonia* cultures was tested (Table 46).

Using the already available TFF samples of S19, S7 and S5 phages, genomic DNA isolation and PCR amplification of the attP site was done. Genomic DNA was isolated from all above TFF phage samples and was found to be above 10kb. attP site amplification was seen by faint bands in S19 phage and the band size was found to be between 500bp and 600bp. The presence of attP site indicated that the phage consisted genes responsible for lysogenic life cycle which indicated that it had the possibility to form prophage with the wilt pathogens and was identified as non-virulent or temperate phage. attP site amplification was not seen S7 and S5 phages which indicate that these phages could be lytic in nature.

R. solanacearum culture was received from ICAR Research Complex for Goa (RS-09-100). Double layer agar method of bacteriophage isolation was carried using RS-09-100 for the phage isolates S1-S22, BW1, TW1 and TW2. Plaque formation was seen in plates inoculated with the following phages i.e. S16, S14, S12, S5, S2, S3, S19, S17, S4, S20, S7, S4, S21 and S22. All the plaques from each phage inoculated plate were carefully scraped out and were stored in TMG buffer at 4°C for overnight phage diffusion to take place. Plaques were centrifuged; the supernatant was filtered and stored in sterile vials at 4° C. Mass multiplication of the S16, S14 and S5 phages has been done using Tangential Flow Filtration method. Then the culture was filtered and the suspension was poured into 250 ml actively growing RS-09-100 culture and kept for overnight incubation in shaker and the next day the culture was centrifuged to remove the bacteria and debris. Then culture was filtered using 0.45µm syringe filters and the resulting suspension was concentrated using 30 Kda cassettes to 50 ml. The phage population in the concentrated sample was 5×10^7 pfu/ml.

Table 46. Isolation of Phages

Ralstonia culture	Bacteriophage culture	Plaque formation
G12	S1-S22, BW1, TW1, TW2	Plaques formation seen with S14, S21, S7, S5, S10, S9, S11, S4 and S3 phages
G8	S1-S22, BW1, TW1, TW2	No plaque formation seen in any plates
G9	S1-S22, BW1, TW1, TW2	No plaque formation seen in any plates
G11	S1-S22, BW1, TW1, TW2	Plaques formation seen in plates inoculated with S8 and S18 phages
G4	S1-S22, BW1, TW1, TW2	No plaque formation seen in any plates
G5	S1-S22, BW1, TW1, TW2	No plaque formation seen in any plates
G6	S1-S22, BW1, TW1, TW2	No plaque formation seen in any plates

ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

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Co-Investigator:
Dr. Amrita Banerjee

a. *RALSTONIA* DIVERSITY

Genomic studies on *R. solanacearum* isolates using PCR based molecular tool was conducted following universal scheme of multi-locus sequence typing (MLST). All the eight genes (*pps*A, *gyr*B, *adk*, *gdh*A, *gap*A, *hrp*B, *fli*C and *egl*) showed amplification in brinjal and tomato isolates from Meghalaya. The amplified product of *fli*C gene of *R. solanacearum* from ginger, capsicum, brinjal and tomato has been sequenced.

b. DIAGNOSTICS

R. solanacearum colonies obtained from Meghalaya were grown on TZC agar medium and confirmed by PCR based detection using genome specific universal primer 759/760. Eight isolates were found to be positive with 282 bp fragment.

c. DISEASE MANAGEMENT

Isolation of new bio-control agent

Burkholderia cepacia (PB2, PBY and PB21) was isolated from soil samples and profiled for antibiotic sensitivity using BD Phoenix 100 (Table 47).

Table 47. Profiling of *Burkholderia cepacia* by using BD Phoenix 100

Antibiotics	PB2 MIC	SIR#	PBY MIC	SIR#	PB21 MIC	SIR#
Amikacin	>3 2	R	>32	R	>32	R
Amoxicillin-Clavulanate	>16/8	R	>16/8	R	>16/8	R
Ampicillin	>16	R	>16	R	>16	R
Aztreonam	4	N	>16	N	>16	N
Cefazolin	>16	R	>16	R	>16	R
Cefepime	16	N	>16	N	>16	N
Cefoperazone-sulbactam	16/8	N	>16/8	N	>16/8	N
Cefotaxime	16	N	>32	N	>32	N
Cefoxitin	>16	R	>16	R	>16	R
Ceftazidime*	4	S	8	S	16	I
Chloramphenicol**	>16	R	16	I	8	S
Ciprofloxacin	>2	R	1	R	2	R
Colistin	>2	R	>2	R	>2	R
Gentamycin	>8	R	>8	R	>8	R
Imipenem	8	N	>8	N	8	N
Levofloxacin***	>4	R	2	S	4	I
Meropenem	2	S	4	S	4	S
Piperacillin	≥4	N	32	N	64	N
Piperacillin-Tazobactam	≥4/4	N	32/4	N	64/4	N
Tetracycline	>8	N	>8	N	>8	N
Trimethroprim-sulfamethoxazole	1/19	S	≥0.5/9.5	S	1/19	S

#Interpretation, S=sensitive, I=insensitive, R=resistant; *PB2 and PBY-sensitive, PB21-insensitive; **PB2-resistant, PBY-insensitive and PB21-sensitive; ***PB2-resistant, PBY-sensitive, PB21-insensitive

Integrated management of bacterial wilt in solanaceous crops

Field trials were conducted for the management of bacterial wilt on different solanaceous crops (capsicum, brinjal, tomato) and ginger using native bio-control agents, *P. fluorescens* (403) and *Bacillus* sp. (507). Among the treatments, combination of *B. subtilis* (BS-2) + *P. fluorescens* (403) + *Bacillus sp.* (507) recorded least infection (16.0%) and highest fresh yield (14.3 t/ha) in capsicum (California Wonder). *B. subtilis* (BS-2) showed effective against brinjal bacterial wilt with minimum infection (47.2%) and highest fresh yield (24.22 t/ha), whereas *P. fluorescens* (403) was found effective for tomato (TO017) with minimum infection (11.1%) and maximum fresh yield (34.9 t/ha) as compared to other treatments (Table 48). In case of ginger bacterial wilt, *T. brevicompactum* + *P. fluorescens* (403) was found effective with minimum infection (22%) and maximum fresh yield (12.3 t/ha). However, the yield loss percentage (16.7%) was found to be lowest with *Bacillus* sp. (507) (Table 49).

Table 48. Efficacy of treatments against bacterial wilt on different solanaceous crops

Treatment	*Capsicum*		Brinjal		Tomato	
	Infection (%)	Fresh yield (t/ha)	Infection (%)	Fresh yield (t/ha)	Infection (%)	Fresh yield (t/ha)
P. fluorescens (403)	16.3	13.9	48.6	23.83	11.1	34.9
Bacillus sp. (507)	16.7	13.7	52.8	22.50	15.3	30.8
P. fluorescens (C- 61)	19.3	12.2	48.6	23.61	18.1	26.7
B. subtilis (BS-2)	19.0	11.8	47.2	24.22	15.3	28.4
Asafoetida + turmeric + water	19.0	12.4	62.5	21.33	15.3	24.9
Asafoetida + turmeric + water + calcium carbonate	18.3	12.7	61.1	21.06	19.4	21.7
B. subtilis (BS-2) + *P. fluorescens* (403)	16.7	13.8	59.7	23.06	15.3	26.6
B. subtilis (BS-2) + *Bacillus* sp. (507)	18.3	13.9	58.3	20.33	20.8	18.3
B. subtilis (BS-2) + *P. fluorescens* (403) + *Bacillus* sp. (507)	16.0	14.3	51.4	22.28	19.4	20.0
Control	22.7	7.2	72.2	12.67	25.0	16.6
SE (d)	1.7	0.3	6.2	0.6	2.0	0.6
CD (p=0.05)	3.9	0.7	14.0	1.4	4.6	1.5

Table 49. Efficacy of treatments against bacterial wilt of ginger

Treatment	Germination (%)	Infection (%)	Fresh yield (t/ha)	Yield loss (%)
P. fluorescens (403)	100.0	36.7	7.5	28.3
Bacillus sp. (507)	100.0	28.3	10.8	16.7
Streptomycin	100.0	31.7	9.5	22.9
Asafoetida + turmeric + water + calcium carbonate	96.7	36.1	6.6	30.3
T. harzianum + *P. fluorescens* (403)	100.0	23.3	7.5	20.4
Asafoetida + turmeric + water	100.0	28.3	11.3	16.8
T. brevicompactum + *P. fluorescens* (403)	98.3	22.0	12.3	17.4
Control	91.7	60.4	8.5	58.5
SE (d)	0.2	0.3	0.8	2.3
CD (p=0.05)	0.4	0.7	1.8	5.5

Effect of *Trichoderma* spp. on growth of ginger and management of bacterial wilt

The effect of eight *Trichoderma* spp. on ginger growth parameters were studied in a pot culture. Among the *Trichoderma* spp. tested, *T. brevicompactum* was found most effective in increasing plant growth parameters followed by *T. asperellum* (Table 50). The *Trichoderma* spp. was also tested for their efficacy against bacterial wilt under pot conditions. Treatment with *T. brevicompactum* showed lowest yield loss (0.56%). The maximum yield (1.15 kg/pot) was recorded with *T. koningiopsis*, which was 219% higher than control. The effect on ginger rhizome development was highest in plants treated with *T. koningiopsis* followed by *T. viride* (Table 51).

Table 50. Effect of *Trichoderma* on ginger growth parameters

Treatment	Plant height (cm)	No. of shoots	Height of shoots (cm)	Shoot diameter (mm)	Leaf total chlorophyll content (µg/g)	No. of leaves in main shoot	Leaf length (cm)	Leaf chlorophyll index (SPAD- 502)
T. viride	79.0	18.0	37.0	7.5	0.8	15.3	19.1	32.7
T. harzianum strain 1	78.0	12.0	42.1	5.7	0.8	15.3	22.0	33.9
T. koningiopsis	74.7	22.0	41.3	5.6	1.1	19.3	21.2	38.1
T. brevicompactum	81.7	24.3	42.1	6.0	1.3	19.7	22.0	38.9
T. longibrachiatum	66.3	14.3	42.1	5.5	0.9	15.3	18.9	35.4
T. virens	62.0	11.0	27.4	4.9	1.1	16.0	21.6	38.3
T. harzianum strain 2	67.0	18.3	36.6	5.1	1.0	16.7	22.0	35.7
T. asperellum	76.7	19.3	42.0	5.9	1.0	15.0	19.7	38.8
Control	59.3	9.3	26.5	4.3	1.1	9.3	18.4	28.1
SE (d)	9.5	8.3	5.5	0.4	0.2	3.7	2.7	5.0
CD (p=0.05)	20.2	17.6	11.7	0.9	0.4	7.8	5.7	10.7

Table 51. Effect of *Trichoderma* on ginger rhizome development

Treatment	No. of Primary finger	No. of Secondary finger	Finger length (cm)		Finger diameter (cm)		Thickness (cm)
			Primary finger	Secondary finger	Primary finger	Secondary finger	
T. viride	23.3	39.7	7.96	1.48	2.13	1.30	2.40
T. harzianum strain 1	15.3	29.0	6.80	1.12	2.00	1.30	2.47
T. koningiopsis	23.7	43.3	7.13	1.68	1.93	1.47	2.50
T. brevicompactum	19.3	37.0	6.97	1.29	2.00	1.37	2.40
T. longibrachiatum	11.7	19.3	5.64	1.21	1.70	1.27	1.97
T. virens	10.0	22.3	6.21	1.26	1.60	1.13	2.13
T. harzianum strain 2	17.3	33.0	6.70	0.91	1.80	1.53	2.33
T. asperellum	9.3	22.3	6.24	1.40	2.13	1.47	2.30
Control	6.0	15.3	4.33	0.78	1.13	0.80	1.17
SE (d)	4.9	9.0	0.25	0.08	0.21	0.27	0.22
CD (p=0.05)	10.4	19.2	0.54	0.17	0.45	0.58	0.46

LIST OF PUBLICATIONS

Research articles

Das A K, Kumar A, Nerkar S & Bawage S 2012 First report of *Phytophthora insolita* in India. Australas. Plant Dis. Notes. 7: 131 – 132. DOI: 10.1007/s13314-012-0066-6.

Das A K, Kumar A, Nerkar S & Bawage S 2012 First report of *Phytophthora lacustris* in India. J. Plant. Pathol. 95 (2): 447.

Dinesh Singh, Yadav D K, Shweta Singha & Upadhyay B K 2012 Utilization of plant growth promoting *Bacillus subtilis* isolates for the management of bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 biovar3. Indian Phytopath. 65 (1): 18-24.

Dinesh Singh, Yadav D K, Shweta Sinha & Harshita Singh 2012 Effect of safe chemicals and bleaching powder on bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 bv. 3. Ann. Plant. Protec. Sci. 20 (2): 426 – 429.

Dubey S C, Priyanka K, Singh, V & Singh B 2012 Race profiling and molecular diversity analysis of *Fusarium oxysporum* f.sp. *ciceris* causing wilt in chickpea. J. Phytopathol. 160:576-587.

Dubey S C, Aradhika T & Singh R S 2010 ITS-RFLP fingerprinting and molecular marker for detection of *Fusarium oxysporum* f.sp. *ciceris*. Folia Microbiol. 55: 629-634.

Durai M, Dubey S C & Aradhika T 2012 Genetic diversity analysis and development of SCAR marker for detection of Indian populations of *Fusarium oxysporum* f. sp *ciceris* causing chickpea wilt. Folia Microbiol. 57: 229-235.

Kumar A, Prameela T P & Suseelabhai R 2013 A unique DNA repair and recombination gene (recN) sequence for identification and intraspecific molecular typing of bacterial wilt pathogen *Ralstonia solanacearum* and its comparative analysis with ribosomal DNA sequences. J. Biosci. 38: 1–12. DOI 10.1007/s12038-013-9312-0.

Mishra R K, Pandey B K, Muthukumar M, Misra A. K, Singh V, Mathew A J, Pathak N & Mohd. Zeeshan 2013 Assessment of genetic diversity of *Fusarium solani* from different agro-ecological regions of India. J. Eco-friendly Agriculture 8 (1): 101-107.

Mishra R K, Pandey B K, Muthukumar M, Mohd. Zeeshan & Pathak N 2013 Detection of *Fusarium* wilt pathogens of *Psidium guajava* L. in soil using culture independent PCR (ciPCR). Saudi J. Biol. Sci. 20 (1): 51-56.

Mishra R K, Pandey B K, Singh V, Pathak N & Mohd. Zeeshan 2013 Genetic characterization of *Fusarium oxysporium* isolated from guava in northern India. African J. Microbiol. Res. 7(33): 4228-4234.

Nath V S, Senthil M, Hegde V M, Jeeva M L Misra R S, Veena S S & Raj M 2013 Molecular evidence supports hyper variability in *Phytophthora colocasiae* associated with leaf blight of taro. Eur. J. Plant Pathol. DOI: 10.1007/s10658-013-0181-z.

Nath V S, Senthil M, Hegde V M, Jeeva M L, Misra R S, Veena S S & Raj M 2013 Genetic diversity of *Phytophthora colocasiae* isolates in India based on AFLP analysis. 3Biotech 3(4): 297-305.

Nath V S, Senthil M, Hegde V M, Jeeva M L, Misra R S, Veen. S S & Raj M 2012 Evaluation of fungicides on Indian isolates of *Phytophthora colocasiae* causing leaf blight of taro, Arch. Phytopathology Plant Protect. DOI:10.1080/03235408.2012.749688

Nath V S, Senthil M, Hegde V M, Jeeva M L, Misra R S, Veen. S S & Raj M 2012 Genetic diversity of *Phytophthora colocasiae* isolates in India based on AFLP analysis. Biotech. DOI 10.1007/s13205-012-0101-5.

Nath V S, Senthil M, Hegde V M, Jeeva M L, Misra R S, Veen. S S & Raj M 2013 PCR-based approach for mining of resistant gene analogues in taro (*Colocasia esculenta*). Arch. Phytopathology Plant Protect. DOI:10.1080/03235408.2012.752608.

Nerkar S G, Bawage S S, Kumar A & Das A K 2012 Unigene-derived microsatellite marker based variation study of *Phytophthora nicotianae* isolates infecting citrus. Indian J. Microbiol. 52(3): 489–491.

Prasath D, Suraby E J, Karthika R, Rosana O B, Prameela T P & M Anandaraj 2013 Analysis of differentially expressed genes in *Curcuma amada* and *Zingiber officinale* upon infection with *Ralstonia solanacearum by* suppression subtractive hybridization. Acta Physiol. Plant. DOI: 10.1007/s11738-013-1362-2.

Ramesh R & Phadke G S 2012 Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by *Ralstonia solanacearum*. Crop Prot. 37:35-41. DOI:10.1016/j.cropro.2012.02.008.

Sudheer Kumar, Deepak Maurya, Shalini Rai, Prem Lal Kashyap & Alok Kumar Srivastava 2012 Computational Mining and Genome Wide Distribution of Microsatellite in *Fusarium oxysporum* f. sp. *Lycopersici*. Not. Sci. Biol 4(4):127-131.

PAPERS PRESENTED IN SEMINARS/SYMPOSIUMS

Achari G A, Ramesh R & Gaitonde S 2012 Diversity, antagonistic and growth promotion ability of xylem residing bacteria isolated from eggplant and chilli. In: Abstracts of ASM 2012, 16-19, June 2012, San Francisco, California USA.

Achari, G A & Ramesh R 2013 Xylem bacteria for the plant growth promotion and pathogen inhibition in brinjal. In: Abstracts of 10th national symposium on biotechnological approaches for plant protection: constraints and opportunities (pp 112-113, 27-29, January 2013, ICAR Research Complex for Goa, Old Goa, Goa.

Anandaraj M & Vinitha K B 2012 Segregation of resistance to *Phytophthora capsici* in *Piper colubrinum*. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management (p.102), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Cissin J, Bhai S R, Nirmal Babu K & Anandara M 2012 Molecular profiling of *Phytophthora* isolates from black pepper using EST-SSR markers. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, Diagnostics and management (p.15), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Das A K 2012 Recent advances in identification and diagnosis of *Phytophthora* spp. with special reference to citrus. In: National symposium on blending conventional and modern plant pathology for sustainable agriculture, 04-06, December 2012, Indian Institute of Horticultural Research, Bengaluru.

Das A K, Ashok Kumar, Nerkar S & Bawage S 2012 *Trichoderma harzianum* strain NRCfBA-44, a novel biocontrol agent for the management of *Phytophthora* root rot in citrus. In: Fifth Indian horticulture congress, 6-9, November 2012, Punjab Agricultural University, Ludhiana, Punjab.

Dinesh Singh, Shweta Sinha, Yadav D K & Mondal K K 2012 Current status on genetic diversity and diagnosis of *Ralstonia solanacearum* inciting bacterial wilt of solanaceous crops in India. In: Abstracts of national symposium on plant microbe interactions and crops health management (p.18), 6-7, October 2012, Visva Bharati, Palli- Sisha Bhawan Institute of Agriculture, Sriniketan.

Dinesh Singh, Shweta Sinha, Yadav D K, Garima Chaudhary & Mondal K K 2012 Genetic diversity of *Ralstonia solanacearum* causing in northern and eastern parts of India. In: Abstracts of national symposium on blending conventional and modern plant pathology for sustainable agriculture (p. 56), 4-6, December 2012, Indian Institute of Horticultural Research, Bengaluru.

Gopalakrishnan C & Rashmi B Artal 2012 Isolation, characterization and management of *Ralstonia solanacearum* E.F. Smith (Yabbuchi *et. al*. 1995) causing bacterial wilt in tomato. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management (p.129), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Gopalakrishnan C & Rashmi B Artal 2012 *In vitro* evaluation of botanicals against *Ralstonia solanacearum* E.F. Smith (Yabbuchi *et. al*. 1995) causing bacterial wilt in solanaceous crops. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management (p.116), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Gopalakrishnan C & Rashmi B Artal 2012 Field efficacy of *Bacillus subtilis* in the management of bacterial wilt in tomato. In: Abstracts of national symposium on blending conventional and modern plant pathology for sustainable agriculture (p.138), 4-6, December 2012, Indian Institute of Horticultural Research, Bengaluru.

Gopalakrishnan C & Rashmi B Artal 2012 Sensitivity of *Xanthomonas axonopodis* pv. *punicae* causing pomegranate bacterial blight to copper, streptocycline and bronopol. In: Abstracts of national symposium on blending conventional and modern plant pathology for sustainable agriculture (p.101), 4-6, December 2012, at Indian Institute of Horticultural Research, Bengaluru.

Johnson George K, Vijesh Kumar I P & Anandaraj M 2012 Transcriptomics approaches for gene discovery in plants - a case study in Piper. In: Agri-2012, International conference on a agricultural & horticultural sciences, 14-15, September 2012, International Convention Center, Hyderabad.

Karthika R, Prasath D, Suseela Bhai R, Leela N K & Anandaraj M 2012 Evaluating the antimicrobial properties of *Curcuma amada* against phytopathogens. In: 6th International symposium on the family zingiberaceae, 10-13, September 2012, University of Calicut, Kozhikode, Kerala.

Karthika R, Suraby E J, Prasath D & Anandaraj M 2012 Isolation and characterization of NBS-LRR-resistance genes in *Zingiber officinale* and *Curcuma amada*. In: 6th International symposium on the family zingiberaceae, 10-13, September 2012, University of Calicut, Kozhikode, Kerala.

Kaushik S K, Reena Sharma, Vinay Bhardwaj, Sanjeev Sharma & Singh B P 2012 Stacking of late blight resistance genes in potato using MAS. In: Proceedings of international conference on plant biotechnology for food security: new frontiers, 21-24, February 2012, National Agricultural Science Centre (NASC), Pusa, New Delhi.

Kumar A, Prameela T P, Birendranath Panja 2012 *Ralstonia solanacearum* causing brown rot of potato in India traces its origin in Race 3/Biovar 2/ Phylotype II strains occurring worldwide: A revelation by Multilocus Sequence Typing (MLST). In: national symposium on blending conventional and modern plant pathology for sustainable agriculture. 4-6 December 2012, Indian Institute of Horticultural Research, Bengaluru.

Manasa M, Gopalakrishnan C, Krishna Reddy M & Riaz Mahmood M 2012 Molecular characterization of *Ralstonia solanacearum* isolates based on rDNA sequence analysis. In: National conference on biotechnology and society – NCBS 2012, 19-20, April 2012, Kuvempu University, Shimoga, Karnataka.

Prameela T P, Suseela Bhai R & Anandaraj M 2012 Isolation of phages infecting *Ralstonia solanacearum* causing bacterial wiltin ginger (*Zingiber officinale* Rosc.) In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management" (p.15), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Prasath D & Anandaraj M 2012 Next generation sequencing for understanding plant pathogen interaction in Zingiberaceae. In: 6th International symposium on the family zingiberaceae, 10-13, September 2012, University of Calicut, Kozhikode, Kerala.

Ramesh R, Achari G & Gaitonde S 2012 Improved detection of *Ralstonia solanacearum* by multiplex and BIO-PCR. In: Abstracts of national symposium on blending conventional and modern plant pathology for sustainable agriculture (pp 76-77), 4-6, December 2012, Indian Institute of Horticultural Research, Bengaluru.

Reena Sharma, Kaushik S K, Bhatt A K, Singh B P, Vinay Bhardwaj, Naresh Thakur & Dalamu 2012 Characterization of potato late blight resistant parental lines by R- gene linked DNA markers. In: Proceedings of national consultation on potato research and development: Way forward (p. 129), 26, September 2012 Orissa University of Agriculture and Technology, Bhubaneshwar.

Rupesh K M, Pandey B K, Amita J M, Neelam Pathak & Mohd. Zeeshan 2012 Nested PCR based identification of guava wilt pathogen *Fusarium oxysporum* f. sp. *psidii* using fungal mycellium as a template. In: International conference on biotechnology: A rendezvous with basic sciences for global prosperity (p.42), 26-27, December, 2012, Pusa campus, Indian Institute for Agricultural Research, New Delhi.

Rupesh K M, Pandey B K, Ashutosh Pandey, Mohd. Zeeshan & Neelam Pathak 2012 Specific detection of *Fusarium oxysporum* f. sp. *psidii* (Fop) causing wilt disease of *Psidium guajava* L. in India. In: National symposium on microbes in health and agriculture (p.87), 12-13, March 2012, School of Life Sciences, Jawaharlal Nehru University, New Delhi.

Rupesh K M, Pandey B K, Muthukumar M, Mishra A K 2012 Development of direct PCR assay for molecular diagnostic of *Fusarium oxysporum* f. sp. *psidii* causing wilt disease in guava (*Psidium guajava* L.). In: National symposium on managing threatening diseases of horticultural, medicinal and field crops (p.102), 3-5, November 2012, Indian Institute of Sugarcane Research, Lucknow.

Sharma I M & Kumar V 2012 Influence of environmental factors on development of grey mould rot in strawberry and its management. In: Emerging issue in plant health management (p.70), 28-29, September 2012, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

Sharma I M 2012 Changing disease scenario in apple orchards of Himachal Pradesh: perspective, challenges and management strategies. In: Proceedings of 6th national seminar on emerging

challenges and paradigm for sustainable agri-rural development (pp 81-82), 18-20, December 2012, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

Sharma I M 2012 Changing disease scenario in apple orchards: perspective, challenges and management strategies. In: Proceedings of national symposium on blending conventional and modern plant pathology for sustainable agriculture" (pp 193-94), 4-6, December 2012, IIHR Bengaluru.

Sharma I M 2012 Prevalence, diagnosis and integrated management of core rot disease of apple fruits: A new emerging disease. In: Proceedings of 6th National seminar on emerging challenges and paradigm for sustainable agri-rural development (p.124), 18-20, December 2012, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

Sharma I M 2013 Diagnosis and integrated management of core rot in apple. In: Proceeding of national symposium crop disease management: Advance and challenges (pp 95-96), 21-23, January 2013, Navsari Agricultural University, Gujarat.

Sharma I M, Negi H S, Gupta Bupesh & Khosla K 2013 Development of eco-friendly integrated management strategy in management of collar rot in apple. In: Proceeding of national symposium crop disease management: Advance and challenges (p.79), 21-23, January 2013, Navsari Agricultural University, Gujarat.

Sharma, I.M. 2012 Changing disease scenario in apple orchards of Himachal Pradesh: probable reasons. In: Emerging issue in plant health management (p.11), 28-29, September 2012, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

Sreeja K, Anandaraj M & Suseela Bhai R 2012 Identification of non-sporulating endophytic fungi with rDNA sequencing. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management (p.44), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Suraby E J, Reena N, NirmalBabu K & Anandaraj M 2012 Isolation and characterization of Resistance gene candidates in *Piper nigrum* L. In: Abstracts of national symposium on heading towards molecular horizons in plant pathology: host resistance, pathogen dynamics, diagnostics and management (p.102), 16-17, November 2012, Sugarcane Breeding Institute, Coimbatore.

Suraby E J, Prameela T P, Prasath D, Nirmal Babu K & Anandaraj, M 2012 Analysis of differentially expressed genes in *Curcuma amada and Zingiber officinale* upon infection with *Ralstonia solanacearum by* Suppression subtractive hybridization. In: Second national symposium on innovative approaches and modern technologies for crop productivity, food safety and environmental sustainability, 19-20, November 2012, Hotel Mangala Towers, Thrissur, Kerala.

Thangavelu R 2012 The status of banana *Fusarium* wilt research in India. In: Abstracts of international banana symposium on banana improvement, health management, use diversification and adaptation to climate change (p.80), 19-22, November 2012, Lees hotel, Kaohsiung city, Taiwan, Republic of China.

Thangavelu R, Gobi M & Mustaffa M 2012 Field evaluation of antagonistic microbes having multiple functions for the suppression of *Fusarium* wilt disease of banana. In: Abstracts of international banana symposium on banana improvement, health management, use diversification and adaptation to climate change (p.69), 19-22, November 2012, Lees hotel, Kaohsiung city, Taiwan, Republic of China.

Vijesh Kumar I P, Johnson George K & M Anandaraj 2012 Studies on the induction of stress responsive genes in *Piper colubrinum* challenge inoculated with *Phytophthora capsici.* In: Plantation Crops Symposium XX (PLACROSYM XX), 12-15, December 2012, Jenneys Residency, Coimbatore, India.

Vijesh Kumar I P, Johnson George K & M Anandaraj 2012 Real time quantitative RT- PCR of osmotin and beta1,3 glucanase genes in *Piper colubrinum* challenge inoculated with *Phytophthora capsici*. In: International conference on advances in biological sciences (ICABS), 15-17, March 2012, Kannur University, Kannur, Kerala.

Vinay Bhardwaj, Reena Sharma, Kaushik S K, Dalamu, Baswaraj Raigond, Sanjeev Sharma & Singh B P 2012 Identification of elite parental lines possessing late blight and potato virus Y (PVY) resistance genes through molecular approaches. In: National consultation on potato research and development: way forward. 26, September 2012, OUAT, Bhubaneswar.

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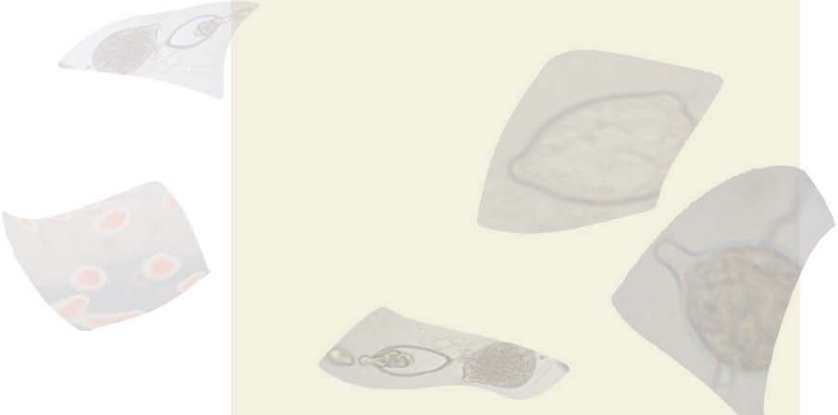
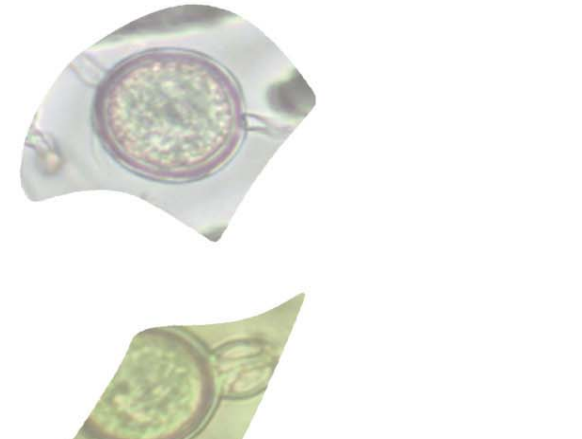
BUDGET (in Lakhs)

Head	Allocation as per EFC (1)	2008-09	2009-10	2010-11	2011-12	2012-13
A. Recurring						
Contingencies/Other charges	725.832	23.85	136.18	241.00	285.9	280.00
TA	124.00	1.00	0	17.00	17.102	1.50
HRD	159.00	0	0	0	0	0
B. Non Recurring						
Equipments	957.00	0	55.35	274.00	426.00	50.00
Total	**1965.832**	**24.85**	**191.53**	**532.00**	**729.002**	**331.50**

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद
भाकृअनुप
ICAR
Agrisearch with a human touch





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AN ICAR OUTREACH PROJECT ON
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