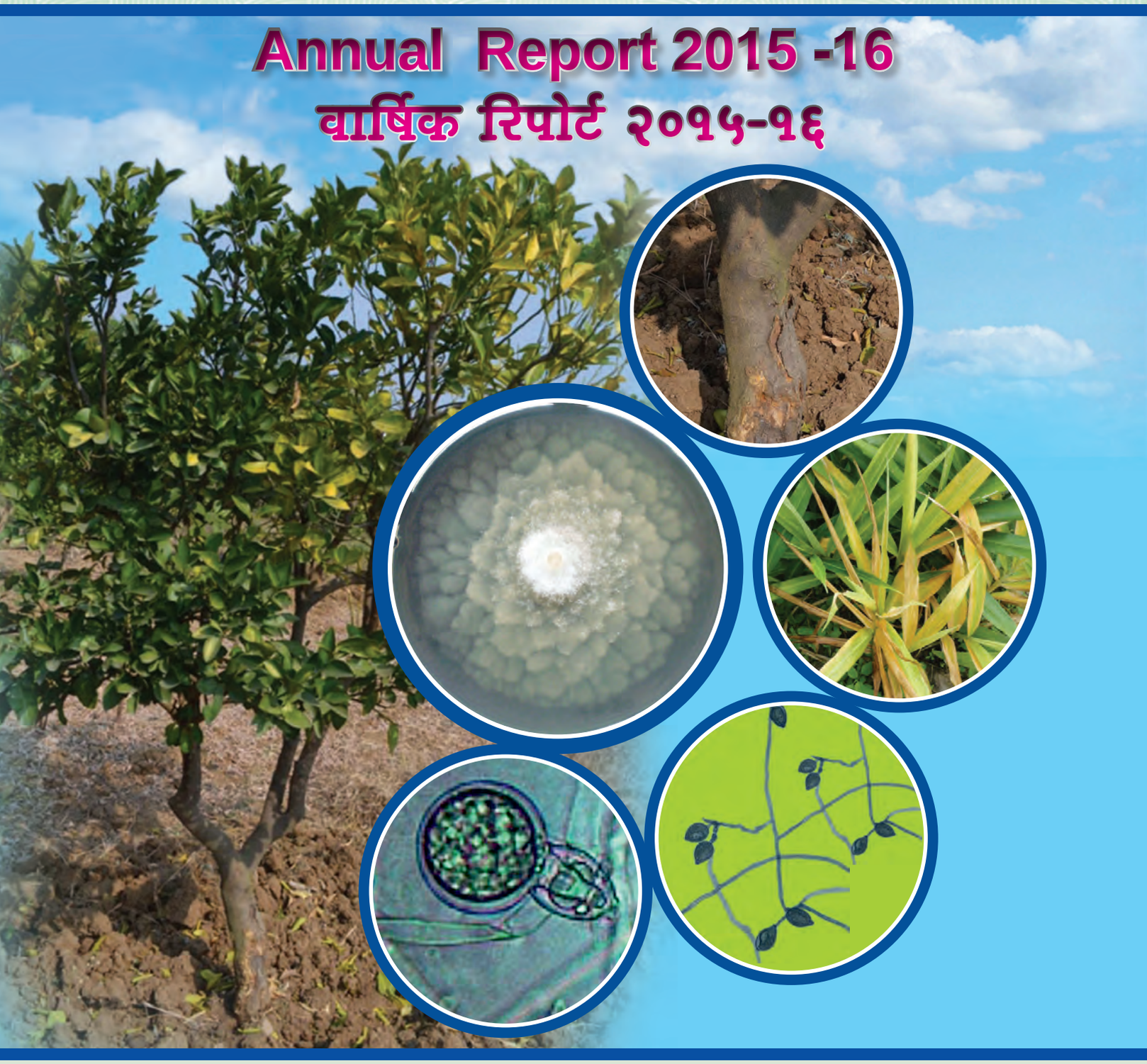
PhytofuRa
AN ICAR NETWORK PROJECT ON
Phytophthora, Fusarium & Ralstonia

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

Annual Report 2015 -16

वार्षिक रिपोर्ट २०१५-१६



ICAR - INDIAN INSTITUTE OF SPICES RESEARCH
P.B. No. 1701, Kozhikode - 673 012, Kerala, India
भाकृअनुप - भारतीय मसाला फसल अनुसंधान संस्थान
पी.बी. नं. १७०१, कोऴिक्कोड- ६७३०१२, केरल, भारत

भाकृअनुप
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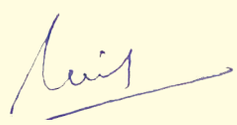
PREFACE

This is the seventh year of the Outreach Project on *Phytophthora, Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops. Launched on 23 February 2009, this novel initiative by Indian Council of Agricultural Research has contributed significantly in understanding deeply the biology, diversity and ecology of these pathogens. The concerted efforts of the 19 partnering institutions have helped in developing out several effective control and management measures to combat them in the field.

During the last year too, a handful of technologies have emerged out of this important project of ICAR. Duplex PCR technique for simultaneous detection of *Phytophthora nicotianae* and citrus greening bacterium, qPCR for detection of *Radopholus similis* in soil and LAMP PCR to detect *Ralstonia solanacearum* are cutting edge diagnostic tools which have very good scope for field deployment. New resistant sources were identified in citrus, arecanut and safflower. Comparative genomics has elucidated the pathogen diversity existing in Indian strains of *Ralstonia solanacearum*. New insights into the interaction of *Phytophthora* with resistant potato variety *Solanum demissum* and black pepper lines, Subhakara and IISR Shakti, was gathered by employing advanced techniques like RNAseq and proteomics.

The Third International Symposium on *Phytophthora* held at ICAR-IIHR, Bengaluru from 09-12 September 2015 and Sixth International Conference on Pathogens, Plants and People organized by Indian Phytopathological Society at New Delhi during 23-27 February 2016 provided ideal platforms to showcase the achievements of the project to the international community. More than 50 papers were presented by the PhytoFuRa investigators in these two events. I am sure that some of these will soon eventually published in leading international journals.

We are into the last year of the project. As there is uncertainty in continued inflow of funds, we need to prioritize the spending. In fact it is time for consolidation and transferring the technologies that emanated for the benefit of the farming community. I take this opportunity to salute the stewardship of by Dr. M. Anandaraj in ably leading the project since its inception and brilliantly coordinating more than 80 scientists across the country. The kind of understanding and supports received from Dr. S. Ayyappan, former Director General, ICAR, New Delhi and Dr. N.K. Krishna Kumar, former Deputy Director General (Hort. Sciences) were unparalleled. We look forward to the wise counsel of Dr. T.Mohapatra, our new Director General, in taking forward this project to a meaningful conclusion. I thank all the directors, investigators, research scholars and other staff for understanding mission and their cooperation and support.



K. Nirmal Babu
Director & Project Co-ordinator (Spices)
ICAR - IISR, Kozhikode

Kozhikode
4th July 2016

EXECUTIVE SUMMARY

PHYTOPHTHORA

DIVERSITY

A total of 182 isolates of *Phytophthora* spp. from Maharashtra, Rajasthan, Arunachal Pradesh, Himachal Pradesh, Uttarakhand, Uttar Pradesh, West Bengal, Tamil Nadu, Karnataka and Kerala were collected and maintained. In India, *P. infestans* populations are polyploids consisting of diploids, triploids and tetraploids and the frequency of different polyploids varied from region to region. *Phytophthora tropicalis, P. capsici, P. palmivora and P. meadii* type cutures procured from American Type Culture Collections (ATCC) were revived and maintained in the National Repository of *Phytophthora* at ICAR-IISR, Kozhikode. ITS phylogeny and MLST analysis of capsici/ tropicalis groups of *Phytophthora* indicated the presence of two separate subclades within Clade 2, deviating from *P. capsici* and *P. tropicalis*. Studies based on SSCP analysis also indicated the existence of inter species hybrids among *Phytophthora* isolates of black pepper. Based on the morphological, physiological and molecular characterization, *Phytophthora* isolates infecting nutmeg could be a hybrid with parentage, *P. citrophthora* x *P. meadii* or *P. capsici* or *P. tropicalis*. Nine different isolates of *Radopholus similis* were collected from black pepper and banana from the surveys conducted in Thiruvananthapuram, Thrissur, Malappuram, Idukki and Wayanad districts of Kerala.

DIAGNOSTICS

Duplex PCR technique for simultaneous detection of *P. nicotianae* and citrus greening bacterium was standardized. Real time PCR based method was developed for the detection and quantification of *R. similis* in soil.

HOST PATHOGEN INTERACTION

RNA sequencing of resistant potato variety *Solanum demissum* using Ion Proton method was attempted for identification of resistance signalling network for late blight resistance and to analyse the transcriptome response to *P. infestans*. Label free proteomics strategy was applied to understand the protein expression and post translational modifications on tolerant (IISR Shakthi) and susceptible (Subhakara) black pepper genotypes. In tolerant genotype the pathogen was suppressed by pattern triggered immunity (PTI) triggered by receptor kinases, where as in susceptible genotype PTI was found to be weak with alteration in host physiology. Salicylic acid mediated resistance was identified during pathogen infection and PR protein production was found to be more in tolerant types.

EPIDEMIOLOGY

Collar rot disease was positively correlated with rainfall, soil moisture and minimum soil temperature and was negatively correlated with maximum soil temperature under nursery conditions. Indirect sporangial germination was observed in *P. infestans* polyploids at lower temperatures of 4 and 8°C, with drastic decrease in germination as the temperature increased to 22°C whereas direct germination was observed in all polyploids from 15°C - 22°C and thereafter it declined drastically. Temperature had an influence on the aggressiveness of polyploids and tetraploid isolates producing larger lesion areas than diploids and triploids.

DISEASE MANAGEMENT

Soil solarization coupled with fumigation with dazomet granules @ 40 g / m^3 of soil during the month of April-May was found effective in reducing soil borne inoculum of *Phytophthora* in citrus nurseries. Demonstration trials on bud rot disease management using *Trichoderma* coir pith cake recorded disease incidence less than 10 per cent. An *in vivo* expression system was developed to produce large amounts of dsRNAs encoding *P. infestans* genes in bacteria and late blight symptoms was lower in potato plants sprayed with bacterial lysate. Plant growth promoting mycorrhizal fungus, *Piriformospora indica*, was found to be an efficient growth promoter for taro varieties and effective in blight management. Under orchard conditions, the addition of cow

urine decoction thrice @7.5% (10L/tree) during March, June and August accompanied with approach grafting and two applications of Curzate (0.3%) during April and August reduced the severity of collar rot.The secondary metabolites extracted from endophytic fungi was found to be inhibitory against *P. capsici.*

GENOMICS

Whole genome sequence of *P. infestans* using Ion Torrent technology was generated with a total of 501,913 raw reads with average read length of 150 bases. Complete mitochondrial genome of A2 mating type has been sequenced and compared with A1 type of *P. infestans.*

FUSARIUM

DIVERSITY

A total of 152 isolates of *Fusarium* spp. isolated from gladiolus, dianthus, chilli, tomato, sugarcane and cumin were purified and maintained. The diversity analysis showed that cox-1 and RPB2 are not suitable markers for grouping of *F. oxysporum* f. sp. *ciceris* (Foc) races representing different geographical regions of India. *F.oxysporum* f.sp. *lycopersici* FOL race 1 is the predominant race infecting tomato in India. Molecular diversity and phylogenetic analysis of *Fusarium* isolates causing wilt of chilli, from various regions of Jammu and Kashmir grouped them into *F.oxysporum* f.sp. *lycopersici* and *F. solani*. Detached leaf assay was standardised for sugar cane to screen *F. sacchari* isolates to understand the variation in pathogenicity.

HOST PATHOGEN INTERACTION

Differential expression analysis of mitogen activated protein kinase gene of Foc infected resistant and susceptible chickpea plants indicated that Foc infection upregulated MAPK gene in the susceptible variety, whereas in the resistant variety, the gene is down regulated. The MAP kinase kinase kinase gene was also differentially expressed at different time intervals in resistant and susceptible chickpea plants. Suppressive subtractive hybridization (SSH) was carried out in banana cv. Grand Naine to identify differentially expressed genes during the interaction of Foc pathogen and the biocontrol agent *T. asperellum*. Real Time PCR analysis of defense related genes in banana indicated that all genes were expressed in the root tissues of plants inoculated with the pathogen and bio control agent.

HOST PLANT RESISTANCE

Varieties of carnation and gladiolus were screened for resistance with fusaric acid extracts. Safflower genotypes DSI-104 and GMU-3263 were found to be tolerant against *Fusarium* wilt. Brinjal grafts raised with root stock of brinjal (EG219) resistant to nematodes, *Fusarium* and bacterial wilt and scion of tomato (IIVR tomato cv. Kashi Amman) resistant to leaf curl virus were popularised in the field.

EPIDEMIOLOGY

Under favourable conditions *Fusarium* wilt inoculum in the affected mother setts act as a source of pathogen inoculum and induced progressive yellowing of leaves, wilting and drying of the plants in all susceptible varieties of sugarcane. Infected soil inoculum played a crucial role in initiation of the disease development as from Tamil Nadu and Bihar. compared to the sett borne inoculum and in susceptible varieties when both sources of inocula are there, disease development is faster.

DISEASE MANAGEMENT

The combined application of *Trichoderma harzianum,* carbendazim and captan was found to be effective for the management of *Fusarium* wilt in gladiolus. *T. harzianum* @ 2ml/kg and cymoxanil 8% + mancozeb 64% @ 0.2% were most effective in managing *Fusarium* wilt in safflower. In tomato, reduction in wilt severity was observed with carbendazim + mancozeb, *Trichoderma* isolates Phyto 6, Phyto 4 and Phyto 9.Liquid formulation of endophytic *Bacillus flexus* and endophytic *Trichoderma asperellum* was effective in wilt suppression and plant growth promotion in banana. Wilt affected sugarcane planting materials treated with fungicide carbendazim

showed improved germination, cane height and cane population than the affected setts. While fungicide treatment improved germination in the disease affected setts the germinated shoots exhibited wilt during grand growth phase of the crop.

GENOMICS

Marker assisted selection was carried out for wilt resistance in interspecific derivatives of safflower. Comparative proteomics study was done using pathogenic and non-pathogenic strains of *Fusarium oxysporum* in banana. Proteomics study was attempted with pathogenic strain of *F. oxysporum* f. sp. *cubense* collected.

RALSTONIA

DIVERSITY

Six isolates of *R.solanacearum* was isolated from chilli and Capsicum grown in Jammu & Kashmir, Meghalaya, Goa and Karnataka. Multi locus sequence typing of three virulence related genes showed that the isolates belong to biovar 3 and 4. *R. solanacearum* strains were collected from diseased tomato plants as well as rhizospheric soil of solanaceous crops from Umiam, Meghalaya. Five new isolates of *R. solanacearum* collected from bacterial wilt infected ginger fields were tested for cross infectivity. Comparative genomics of 10 isolates of *R. solanacearum* infecting ginger, tomato, potato and brinjal from different locations of India showed that gene families were conserved among these ten strains and also identified unique genes present in each strain, which confirms that strains are much more diverse among themselves.

DIAGNOSTICS

LAMP PCR was standardized to detect *R. solanacearum* infecting brinjal from soil and plant tissues. A strain specific and sensitive detection methodology using Real Time Loop Mediated Isothermal Amplification (Real Time-LAMP) was developed for identification of race 4 strain ginger.

HOST PLANT RESISTANCE

The potential bioagents, *P. fluorescens* and *B. subtilis* were used alone and in combination with *R. solanacearum to* understand the induction of defense related proteins in tomato.

HOST PATHOGEN INTERACTION

The *R. solanacearum* responsive gene PAP2 was randomly cloned into the pTRV2 vector and transformed into *Agrobacterium tumefaciens*, and then inoculated into potato variety, Kufri Jyoti to create VIGS plants. VIGS plants that barely showed wilting symptoms after inoculation with the pathogen *R. solanaceraum* and the resistant plants showed accelerated cell death and rapid accumulation of reactive oxygen species.

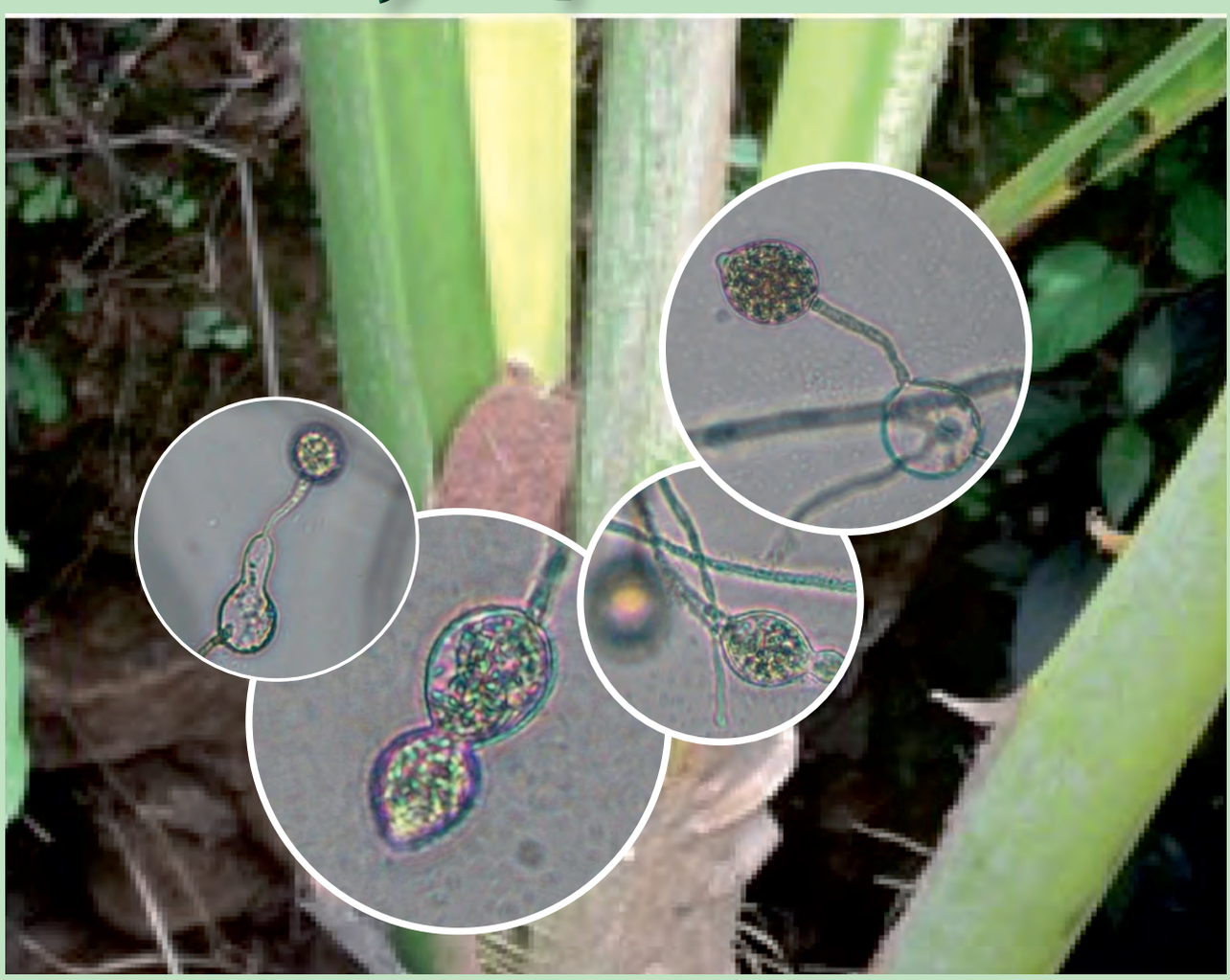
DISEASE MANAGEMENT

Brinjal grafts with cultivated brinjal and wild brinjal (Agassaim and Taleigao) was found to be completely resistant to bacterial wilt. Grafted plants produced fruits with same size, colour and same number of fruits per plant as that of fruits in non grafted seedling plant. Four strains of xylem residing bacteria and two strains of endophytic bacteria were identified as effective in managing brinjal wilt under greenhouse conditions. Drenching tomato with biocontrol agents *P. fluorescens* and *B. amyloliquefaciens* was effective for the management of bacterial wilt disease. Apoplastic bacteria, IISR GAB 107 was effective in reducing bacterial wilt in ginger and the bacteria effectively colonized the rhizosphere and apoplastic fluid of pseudostems and leaves of ginger plants.

GENOMICS

Resistant and susceptible brinjal varieties were used to develop the complete transcriptome data to identify the genes involved in resistant reaction during the infection process of *R. solanacearum.* Two isolates of *R. solanacearum* Biovar 3 and 4 infecting ginger *viz.,* GRs Sikkim and GRs Mep2 were whole genome sequenced. The genomes were mined for various effectors proteins and other virulence factors.

Phytophthora



ICAR – CENTRAL CITRUS RESEARCH INSTITUTE NAGPUR

Principal Investigator:
Dr. A K Das

Co-Investigator:
Dr. I P Singh

A. DIVERSITY

Collection and conservation of *Phytophthora* spp isolates infecting citrus

A total of 37 *Phytophthora* spp. isolates (27 isolates of *Phytophthora nicotianae,* 5 isolates of *P. palmivora*, 3 isolates of *P. heveae,* 1 isolate of *P. frigid* and 1 isolate of *Phytopythium vexans)* were isolated and purified from soil, root, leaf, fruit and water samples collected from citrus orchards situated in Vidarbha region of Maharashtra, Pratapgarh District of Rajasthan and four districts (Lohit, Changlang, Namsai and East Siang) of Arunachal Pradesh (Fig. 1a, 1b and 1c). These isolates are being maintained in sterile distilled water and in corn meal agar plates in a BOD incubator at 25°C. *P. frigida* was isolated from a declined *Cassia fistula* tree (non-citrus host) and a *P. palmivora* isolate from an infected cocoa fruit. These isolates are also maintained in the repository of *Phytophthora* spp.

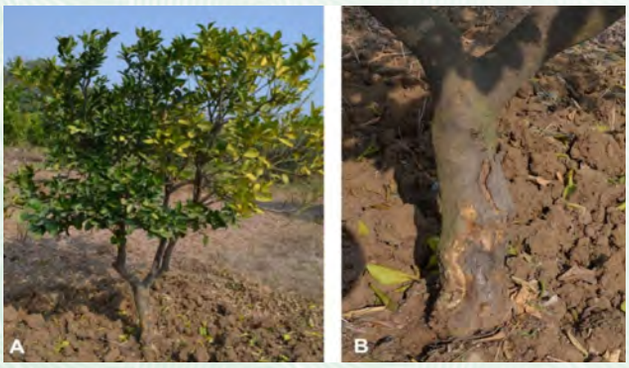


Fig.1 a. Foot rot of Mosambi orange
A. Yellowing of leaves B. Girdled tree trunk

Fig .1 b. *Phytophthora* induced gummosis in Nagpur mandarin

Fig. 1 c *Phytophthora* induced brown rot of Mosambi sweet orange fruits

Morphological studies

Sporangial morphology of 37 isolates (NRCPh 201–237) using agar disk in water technique showed that the sporangia of *P. nicotianae* isolates were ovoid to globose with prominent papillae and non-caducous and *P. palmivora,* sporangia were variable in shape, mostly ovoid to globose, limoniform with prominent papillae, short pedicel and caducous. Sporangiophore showed simple sympodial branching. Out of 54 *Phytophthora* spp. isolates (NRCPh-184 – 237) 42 were found to be A1 mating types, 1 isolates was A2 mating type, 5 isolates were homothallic, 4 isolate were acting as A1 mating type and 2 isolates found to be inducing oospores in A1. All 3 isolates of *P. heveae* showed papillate sporangia with variable shapes but

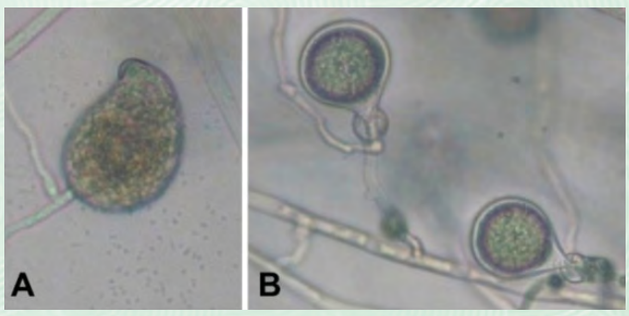


Fig. 2 A. Sporangial morphology of *P. heveae*
B. Oospore morphology of *P. hevea*

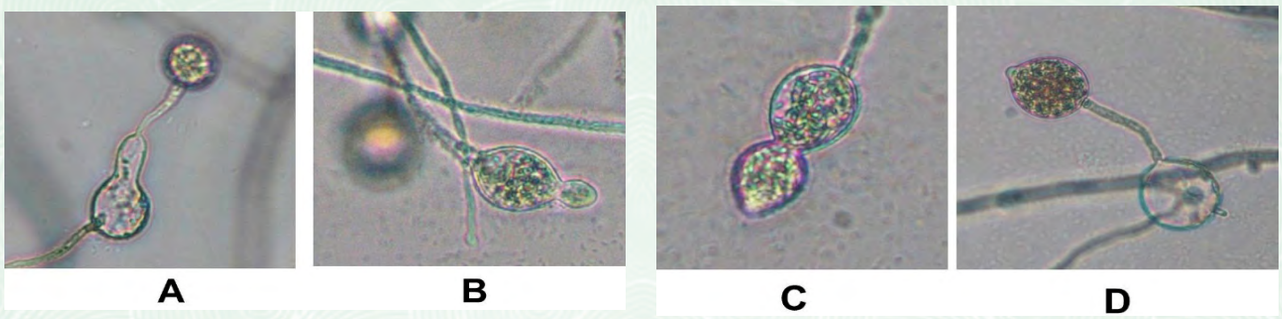


Fig. 3 Morphology of *Phytopythium vexans* (A, B, C, D)

most prominently found to be ovoid with few having asymmetric attachment. The isolates were homothallic with smooth walled oogonia with tapered stalks and aplerotic oospores (Fig. 2). Sporangial morphology of *Phytopythium vexans* showed the characters of *Phytophthora* and *Pythium* (Fig. 3).

Metalaxyl sensitivity

Metalaxyl sensitivity was determined by growing the isolates on corn meal agar (CMA) amended with fungicide metalaxyl at different concentrations *i.e.*1 ppm, 5 ppm, 10 ppm and 50 ppm . Among the 54 isolates, eight moderate isolates (M) that exhibited growth greater than 40 per cent on media amended with 5 µg ml-1 of that on non amended media and 46 isolates were found to be sensitive. None of the isolate was found to be resistant against metalaxyl.

Molecular diversity analysis

ITS-RFLP analysis of the extracted DNA (with the restriction enzymes MspI and AluI) of 42 *Phytophthora* spp. isolates (NRCPh 196-237), showed that the isolates include *Phytophthora nicotianae, P. palmivora, P. citrophthora, P. heveae* and *P. frigida.* A total of 8 ITS sequences, one EF-1α, one Cox-I, and 3 epiC1 gene sequences were submitted for 11 *Phytophthora* spp. isolates at the NCBI GenBank database.

B. DIAGNOSTICS

Duplex PCR for detection of *P. nicotianae* and citrus greening bacterium

duplex PCR technique for simultaneous detection of *P. nicotianae* and citrus greening bacterium was standardized using pure samples. Optimization of duplex PCR to detect the pathogens in the fibrous roots is in progress.

C. HOST PATHOGEN INTERACTION

Identification of differentially expressed genes during confrontation of *Trichoderma harzianum* and *Phytophthora nicotianae:*

Preliminary attempts were made towards exploring the differential expression of mycoparasitism related genes *viz.,* asm (acid sphingomielinase), bgn (ß-1,3-endoglucanase), chit (chitinase 33), endo (endochitinase 42), exo (exo-rhamnogalacturonase), gly (glycosyl hydrolase), chk (checkpoint-like protein), ser (serine threonine-protein kinase), ptr (peptide transporter), and aqp (aquaporin) genes.

Fig. 4 Smooth Flat Seville found tolerant to *P. nicotianae*

D. HOST RESISTANCE

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

In the rootstock screening experiments, Smooth Flat Seville (SFS) and x Argentina trifoliate orange were found tolerant against *P. nicotianae* infection. Smooth Flat Seville (SFS) (Fig. 4) is a sour orange hybrid (Citrus x Aurantium) which can be used as alternative to conventional rootstocks like rough lemon and Rangpur lime.

E. DISEASE MANAGEMENT

Integrated management of *Phytophthora* diseases infecting citrus

Soil solarization coupled with fumigation with dazomet granules (Basamid, obtained from M/S Margo Pvt. Ltd., Pune) @ 40 g / m^3 of soil and covered with polythene sheet during the month of April-May was found effective in reducing soil-borne pathogen inoculum in nursery soil.

Talc based formulation of *Trichoderma harzianum*

Talc-based formulation of *T. harzianum* (strain NRCfBA-44) has been developed (Fig. 5). The formulation reduces the number of *Phytophthora* propagules in nursery soil as well as orchards. The formulation also enhances the growth parameters of citrus seedlings in the nursery.

Fig. 5 Talc-based formulation of *T. harzianum* (strain NRCfBA-44)

Integrated management of *Phytophthora* root rot and gummosis in Nagpur mandarin

Field trials undertaken in Nagpur mandarin orchard showed that integrated application of Bordeaux Paste+ *Trichoderma harzianum* Str. 44 + Fosetyl Al found to reduce gummosis lesion numbers and also the number of *Phytophthora* propagules.

ICAR-CENTRAL PLANTATION CROPS RESEARCH INSTITUTE, KASARAGOD

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Co-Investigators :
Dr. Prathibha V H
Ms. Chaithra M.

A. DIVERSITY

PCR based detection of *Phytophthora* species

Three species, *P. meadii, P. citrophthora* and *P. colocasiae* of Clade II with same band size were subjected to high Resolution melt (HRM) curve analysis. Difference was observed among *P. meadii, P. citrophthora* and *P. colocasiae,* in A/T and C/A allele which consistently separated the three species. The initial melt curves differentiated each species from the other by small shift in peaks developed. All the three species displayed a single melting peak (Fig. 6).

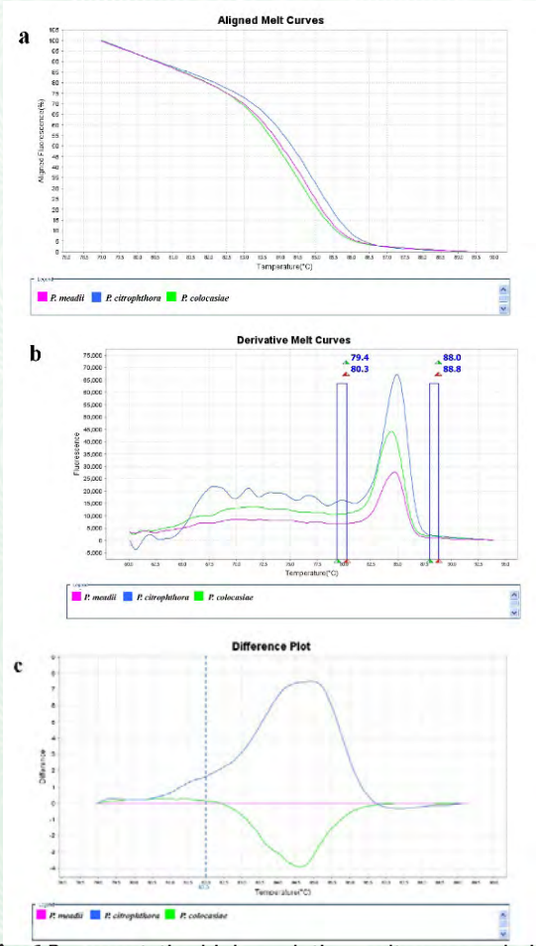

Fig. 6 Representative high resolution melt curve analysis (HRM) curves and difference plot.

B. HOST PLANT RESISTANCE

Seven arecanut varieties (Mangala, Sumangala, Sreemangala, Swarnamangala, Mohitnagar, Nalbari, Srivardhana), a dwarf mutant (Hirehalli dwarf) and two wild types (*Areca triandra* and *Areca concinna*) were screened against *Phytophthora meadii* under *in vitro* conditions by detached arecanut method. Except the wild types all other genotypes (Fig. 7) showed typical symptoms of fruit rot disease three days after inoculation.

Fig.7 Symptoms expressed on wild types

C. DISEASE MANAGEMENT

Isolation of endophytes and testing against *Phytophthora* spp.

Five endophytic *Trichoderma* isolates from cocoa were evaluated against three virulent *Phytophthora palmivora* isolates infecting both cocoa and coconut by dual culture method. Isolate CPCRI-COC-3 recorded complete inhibition of mycelial growth within three days after inoculation.

Field trials on management of coconut bud rot disease using new fungicides

Field trial on management of coconut bud rot was conducted in Konnakkad of Balal panchayath with nine treatments after recording pretreatment disease incidence of 48.5 per cent. Among the tested treatments no disease incidence was recorded in the treatments *viz.,* chlorothalonil, iprovalicarb 5.5 +

propineb 61.25 % WP, dimethomorph 50 WP, Fosetyl-AL 80 WP + propineb 61.25%WP and Metiram 50 + pyraclostrobin 50%WG . Whereas, fresh bud rot disease incidence (3.3 per cent) was recorded in the treatments copper hydroxide 77%WP, copper oxychloride 50%WP, *Trichoderma* coir pith cake and in standard check 1% Bordeaux mixture.

Demonstration trials on bud rot disease management

Demonstration trial on bud rot disease management initiated during 2013 at Manjeshwar and West Eleri panchayaths using *Trichoderma* coir pith cake were evaluated for post treatment bud rot incidence (Fig.8a & b). During 2014, the disease was not recorded in Manjeshwar whereas 8.7 per cent incidence was recorded at West Eleri. During 2015, bud rot incidence recorded in Manjeshwar and West Eleri panchayaths were 1.1 and 6.2 per cent respectively.

Fig. 8 a *Trichoderma* coir pith cake

Fig. 8b *Trichoderma* cakes kept in the innermost leaf axil

Field trials on management of fruit rot disease

Field trials on the management of fruit rot disease of Arecanut with thirteen treatments were conducted in CPCRI, Regional Centre, Kidu, and CPCRI, Regional Station, Vittal. Disease incidence was noticed from 2nd week of July in Kidu but no incidence was recorded in Vittal trial throughout the season. In Kidu, the disease incidence recorded in Mandipropamid sprayed plots was 23.3 per cent followed by 61.25 per cent in the treatments Fosetyl-Al 80WP+Propineb WP and 1% Bordeaux mixture .

ICAR-CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA

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Dr. S K Chakrabarti (w.e.f 27.01.2016)

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A. DIVERSITY

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

Infected potato samples were collected from different geographic locations *viz.* Himachal Pradesh, Uttarakhand, Uttar Pradesh and West Bengal. Over 120 isolates of *P. infestans* were purified and maintained on Rye Agar Media.DNA was extracted from 180 isolates of *P. infestans.*

Determination of ploidy status of *P. infestans* population

In continuation to last year's study, a total of 130 *P. infestans* isolates collected from Himachal Pradesh (north western hills), Meghalaya (north eastern hills), The Niligiri (southern hills), Karnataka (southern plateau), Punjab, western Uttar Pradesh, Bihar, Madhya Pradesh and West Bengal (Indo Gangetic plains) were characterized for ploidy status using Feulgen cytophotometry. Results revealed that *P. infestans* population in the country are polyploid consisting of diploids, triploids and tetraploids and the frequency of different polyploids varied from region to region. In north western hills, the frequency of diploids was highest (90.48 per cent) followed by triploids and tetraploids (4.76 per cent), whereas in north eastern and southern hills, frequency of diploids was highest (80 per cent) followed by triploids (20 per cent). Tetraploids were not present in north eastern and southern hills. In Indo Gangetic plains, the frequency of diploids, triploids and tetraploids were 87.88, 9.09 and 3.03 per cent, respectively, but only diploids were recorded in plateau.

Genotyping of *P. infestans* (Indian population) using SSR markers

A sub-set of core collection of *P. infestans* isolates of Indian population were genotyped using four polymorphic SSR primers (Pinf SSR1, SSR2, Pi4B and Pi04). Most of isolates showed two alleles (228 and 243 bp) and some showed four alleles (213, 229, 238 and 245 bp) with primer Pinf SSR1 in 100 isolates of *P. infestans*. Whereas, two alleles (198 and 204 bp) were observed with primer Pi4B in 50 isolates of *P. infestans* and three alleles were observed with primer Pi04 (164, 172, and 186 bp) in 50 isolates of *P. infestans.* Mostly two alleles (170 and 184 bp) were observed with primer Pinf SSR2 but in some of isolates more than two alleles were observed.

Mitochondrial haplotyping for lineage determination

A sub-set of population comprising 82 isolates collected from Himachal Pradesh, Punjab, Uttar Pradesh, Bihar, Meghalaya and West Bengal were analyzed with primer F2-R2 (digested with MspI) and F4-R4 (cut with EcoR1) for mitochondrial haplotyping. The results revealed that all the isolates belonged to the Ia haplotype (Fig. 9) confirming the last year results.

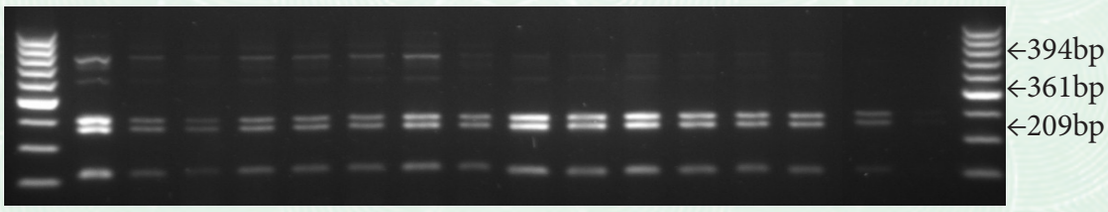


Fig. 9 Mt haplotyping with primer F4-R4 Lane: 1-15 *P. infestans*

B. EPIDEMIOLOGY

Effect of temperature on sporangial germination of polyploids

Sporangial germination of polyploids was studied at 4, 8, 15, 18, 20 and 22°C. After 2, 4, 6 and 24 h the germination (direct, indirect and total) was assessed by observing microscopically. Significant differential effect of temperature on sporangia germination for the polyploids was observed. At 4 and 8°C, the indirect germination was highest with drastic decrease as the temperature increased to 22°C. Direct germination was observed in all polyploids from ≥15°C and up to 22°C and thereafter it declined drastically. Over all temperatures, and total germination percentage were same for diploids and tetraploids (62.38 per cent) as compared to 56.38 per cent in triploids.

Maximum relative rate of sporangial germination (indirect) in diploid was recorded at 8°C (0.063 per hour) followed by 4, 15, 18 and 20°C in descending trend, whereas rate of direct germination increased with increase in temperature up to 22°C. In triploids, maximum relative rate of indirect and direct germination recorded at 8°C and 15°C were 0.025 and 0.044, respectively and then decreased with increase in temperature. In tetraploids, relative rate of indirect germination increased with increase in temperature up to 15°C and then declined with further increase in temperature. On the other hand, highest rate of direct germination was recorded at 20°C followed by 18 and 15°C temperature.

Effect of temperature on aggressiveness of polyploids

Polyploids were studied for their comparative aggressiveness using detached leaf of cv Kufri Bahar. The inoculated leaves were incubated in plastic trays at 10, 15, 18, 20, 24 and 28°C and assessed for incubation period (IP), latent period (LP), lesion area (LA), and sporulation. In general, IP decreased with increase in temperature and least IP for all polyploids occurred at 24°C. The IP was least for tetraploids with a mean of 74 h compared to 84 h for triploids and 91.2 h for diploids. Lesion area increased with increase in temperature in all polyploids up to 20°C and thereafter it decreased. Over all temperatures, tetraploids isolates had larger lesion areas than diploids but did not differ significantly, whereas LA differed significantly from triploids. Temperature differentially affected sporulation of all polyploids, and the highest sporulation recorded at 15 and 18°C. Polyploids did not sporulate at 10°C and 28°C. Diploids and triploids did not sporulate at 24°C. In general, diploids had highest sporulation followed by tetraploids.

Effect of different temperature on new and old population of *P. infestans* on epidemiological components

The study conducted comprising of 17 isolates of *P. infestans* from Uttar Pradesh (5), Uttarakhand (3), Karnataka (4), and Himachal Pradesh (5) on effect of temperatures on epidemiological parameters of *P. infestans* showed that the incubation period (IP) was 37.35 h and latent period (LP) was 43.90 h at 22°C whereas at 25°C, IP was 44.38 h and LP was 53.70 h, respectively. When, same population was further tested at higher temperature *i.e.,* 28°C and 30°C, IP and LP were increased. At 28°C, *P. infestans* population of UP, HP and Uttarakhand showed higher lesion area than the Karnataka isolates.

Effect of elevated temperature on host resistance against *P. infestans*

Four late blight differentials (R1, R2, R7& R9) and one cultivar (Kufri Bahar) were grown at 20°C, 25°C and 28°C and then detached leaves were challenge inoculated with *P. infestans*. At higher temperature (25°C) only in R7 differential large lesion size (1.78 cm^2) was observed compared with 20°C (1.5 cm^2).

C. HOST PATHOGEN INTERACTION

RNA sequencing of *Solanum demissum* for identification of resistance signalling network

RNA sequencing of *S. demissum* using Ion Proton for identification of resistance signalling network for late blight resistance was attempted to analyse the novel transcriptome response to *P. infestans*. Expression profiles obtained from read mapping of the *S. tuberosum* reference genome and the *S. demissum* transcripts revealed a differential response to the *Phytophthora,* with 651 (at 24 hpi), 989 (48 hpi) and 926 (at 96 hpi) differentially expressed genes *viz.,* cellular and signalling network genes, like ubiquitin mediated F-box protein, cellular response, resistance related metabolites as well as R genes and pathogenesis related genes.

D. GENOMICS

Preparation of multi si RNA vector for multiple gene knock-down applications for late blight resistance

Using siRNA target finder software, the effective siRNA of the targeted gene of interest (sporulation, signalling process and protein synthetic machinery) were selected and stacked in a single synthetic construct driven by T7 promoter on both 5' and 3' end. The efficacy of siRNA has been assayed/checked using *in vitro* ds RNA technology through detached leaf assay against *P. infestans*.

Comparative genome analysis of Irish Famine pathogen with Indian A2 type *Phytophthora infestans*

Whole genome sequence of *P. infestans* (S15) using Ion Torrent (Next Generation Sequencing) technology was generated with a total of 501,913 raw reads with average read length of 150 bases. Using fastaq format of 20X genome data of Indian isolate and using Varscan, SNP data at genome level was generated with 7,342 SNPs at 20X coverage. INDELs data showed 743 at 20X coverage and 4,504 at 10 X coverage that confirmed existence of variation between Indian and European isolate. The result is being used for isolation of effector genes and targeted re-annotation of novel effector genes. *P. infestans* S15 novel genes were compared with sequence of *P. infestans* strain T30-4, which has been sequenced by Broad Institute, USA.

Mitochondrial genome mapping of *Phytophthora infestans* A2 mating type and comparison with A1 type

Complete mitochondrial genome of A2 mating type has been sequenced which is slightly higher than all A1 type and showed 99.5 and 99.4 per cent homology with Ia and Ib type, respectively and 94.2 and 94.3 per cent with IIa and IIb, respectively with A1 mating type.

Silencing of transposable elements from *P. infestans* to reduce pathogen dynamism

Nearly 33 per cent of the *P. infestans* genome is covered with transposable Long Terminal Repeats LTR/Gypsy-Ty3 element, which is almost 1/3rd of the genome. RNAi construct was designed to silence the LTR/Gypsy-Ty3 element in an attempt to reduce the *P. infestans* dynamism. A total of 300 stem cuttings of Kufri Bahar (A LB susceptible cultivar) have been used for transformation and kept on MS media along with Kanamycin selection. The cut ends of the approximately 200 stem cuttings have developed callus and initiated shooting.

E. HOST PLANT RESISTANCE

Allele mining for late blight resistance genes in potato wild species

The highly resistant species were selected namely *S. berthaultii, S. cardiophyllum, S. iopetalum, S. jamesii, S. lesteri, S. microdontum, S. pinnatisectum, S. polyadenium, S. polytrichon, S. trifidum* and *S. verrucosum*. The potato R genes (Rpi-pta1, Rpi-edn1.1, EDNR2GH7, Rpi-hjt1.1, SNKR2GH5, Rpi-snk1.1, Rpi-blb1/RB, Rpi-vnt1.1, Rpi-bt1, Rpi-sto1, Rpi-blb2, RGA1, RGA3 and RGA4) conferring resistance to late blight were used for allele mining into these wild species.

Cloning of late blight resistance genes from wild potato species

Allelic variants of the late blight resistance gene Rpi-pta1 (2.5 kb) was isolated from the late blight resistant wild potato species *S. cardiophyllum*. This novel gene was cloned in a binary vector pRI101AN at the restriction sites of Kpn I-EcoRI. Further, the gene insert was confirmed by colony PCR and restriction digestion.

Molecular diversity analysis of the wild potato species by SSR markers

A total of 84 wild potato species/accessions were analyzed for molecular diversity using 14 SSR markers and results revealed diversity among the wild species that are useful for exploitation in potato. A similarity matrix was calculated by the Jaccard's coefficient and dendrogram was constructed using unweighted pair-group method (UPGMA) clustering method with the program DARwin (Fig. 10).

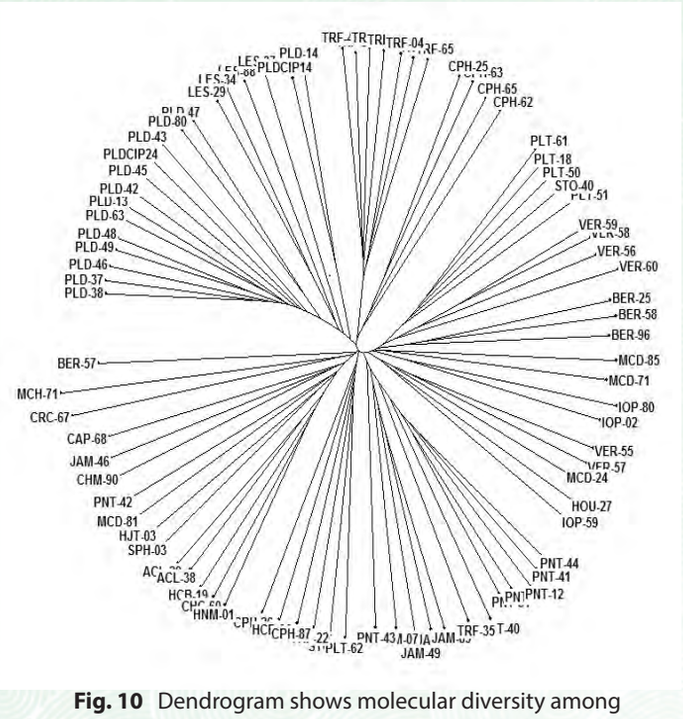


Fig. 10 Dendrogram shows molecular diversity among the wild potato species analyzed by SSR markers.

Identification of genotypes with multiple resistance genes

Hybridization was done to combine multiple resistance genes in a common genotypic background mainly for late blight (R1 and R3a), PVY (Ryadg, Rysto) and potato cyst nematodes (H1, HC_QRL and Gro1-4) resistances using molecular markers. A total of 47,347 hybrid TPS were extracted from 26 successful crosses. The seedlings of about 6000 TPS belonging to 8 crosses were raised and about 50 F1C1 clones were selected based on the agronomic traits and late blight resistance. About 52 clones were advanced to F1C2 generation based on late blight resistance and phenotypic traits. In F1C3 generation 22 clones were identified with stacked R genes for late blight resistance . Two recently imported novel late blight and virus resistant genotypes *viz.,* Toluca (blb2) from The Netherlands and Sarpo Mira (R3a, R3b, R4, Ny-Smira, Rpi-Smira1and Rpi-Smira2) from Denmark were validated for the presence of respective genes.

DISEASE MANAGEMENT

A total of 31 bacterial isolates were purified and screened for their biocontrol activity against *P. infestans* using dual culture test. Six bacterial isolates showed positive inhibition activity against *P. infestans* which ranged from 48.15 to 92.59 per cent inhibition. The detached leaf assay revealed that TP08 (*Pseudomonas fluorescens* isolate) was effective in inhibiting lesion area development when compared with *P. aeruginosa*.

Testing of rhamnolipid based formulation against *P. infestans* under field condition

A combination of rhamnolipid + botanical was tested under field conditions in Modipuram against standard contact fungicides. The minimum disease severity of 61.7 per cent was observed in chlorothalonil treated plots followed by propineb (65.0%), botanical+rhamnolipid (68.3%), mancozeb (70.0%), rhamnolipid (71.7%) and botanical (75.0%) compared with control at 100 days of crop stage.

dsRNA based fungicide for management of potato late blight

Developed an *in vivo* expression system to produce large amounts of dsRNAs encoding *P. infestans* genes in bacteria with a view to provide a practical control of late blight disease in potato. Late blight symptoms were significantly lower when plants were sprayed with bacterial lysate before pathogen inoculation (Fig. 11) .

Without dsRNA **With dsRNA**

Fig. 11 Screening of ds RNA against Late blight

ICAR-CENTRAL TUBER CROPS RESEARCH INSTITUTE, THIRUVANANTHAPURAM

Principal Investigator:
Dr. M L Jeeva

Co-Investigators:
Dr. R S Misra (till July, 2015)
Dr S S Veena

A. DIVERSITY

Isolation and characterization of *P. colocasiae* isolates

Fifteen *P. colocasiae* isolates were added to the existing culture collection. These isolates were used for studying yearly variation in *P. colocasiae* isolates from the same field and confirmed to the species level using species specific PCR . A total of 40 *P. colocasiae* isolates collected from different taro growing regions of India, maintained at ICAR-CTCRI were used for genetic diversity analysis using SRAP markers. Nine primer combinations amplified 118 reproducible fragments ranging in size from 200 to 1800 bp, of which 118 were polymorphic (100 %) with some bands common to majority of isolates, while others were unique to one or few isolates (Fig.12). Forty *P. colocasiae* isolates were also used to amplify the Larger Sub Unit (LSU) region and the sequence analysis revealed 98–99 % nucleotide sequence similarity to each other and 97–99 % similarity among the isolates of *P. colocasiae* available in the GenBank database.

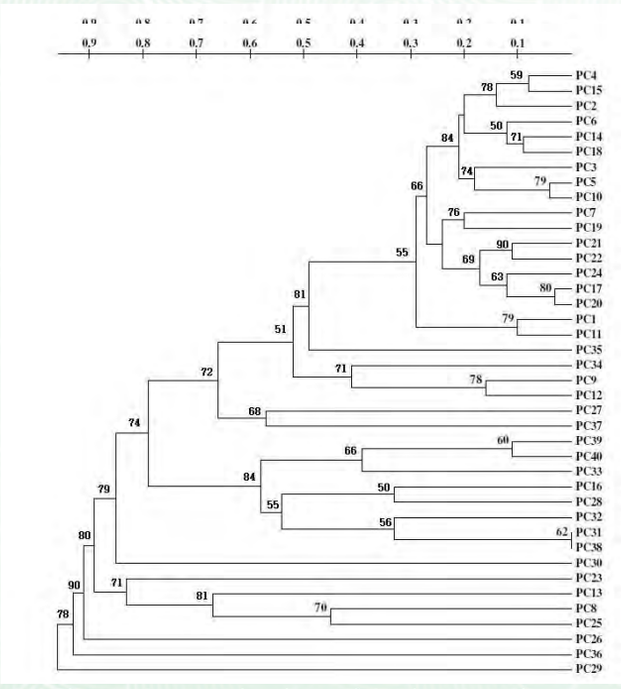


Fig. 12 Genetic relatedness of *P. colocasiae* isolates as revealed by SRAP markers

B. DIAGNOSIS

qPCR based resistance screening of taro

Towards developing a relative quantification assay for the accurate detection of pathogen load in the sample, different genes (actin, GAPDH, ubiquitin and EF1) are being evaluated to find a suitable reference gene for the performing the assay.

C. GENOMICS

SSR genotyping of *P. colocasiae*

Totally, 40 *P. colocasiae* isolates obtained from different regions of India were used for the study and identified suitable SSR markers for characterizing *P. colocasiae* from reported SSR markers for *Phytophthora* sp . The coefficient of genetic differentiation among populations (GST) was 0.1896, which supports the AMOVA analysis indicating only limited genetic diversity among populations and high diversity within populations. The estimate of gene flow (Nm) among populations was 2.13 migrants per generation.

D. HOST RESISTANCE

Identification and characterization of resistant gene analogues (RGAs) in taro

The putative RGA sequences were amplified using degenerate primers targeting the region from the P-loop and GLPL (A/T) L motif of the NBS region of RGAs from resistant (Muktakeshi) and susceptible (Sree Kiran) taro cultivars. The phylogenetic study grouped the taro RGAs into single cluster irrespective of the resistant and susceptible variety showing that there is no variation in the genomic region of the resistant and susceptible variety.

Field screening of taro accessions against leaf blight disease

Taro accessions were screened under field conditions (212 nos) and in grow bags (19 nos) for leaf blight incidence. Among the 212 accessions screened, 18 were found to be susceptible (S), 56 were highly susceptible (HS) and 138 accessions were tolerant (T) to leaf blight disease. Under grow bag conditions, three accessions showed consistently moderate resistant reaction.

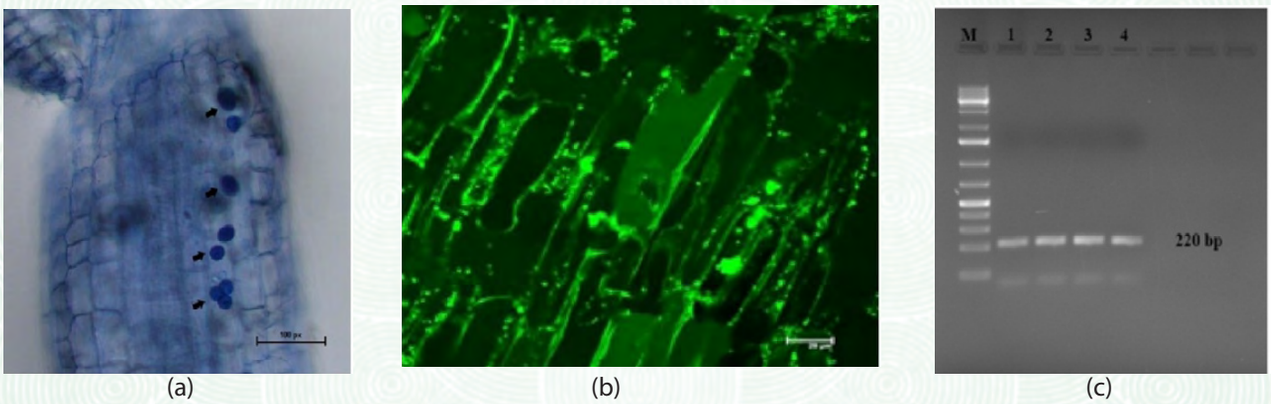
E. DISEASE MANAGEMENT

Field evaluation of the effective biocontrol agent

The most effective bio control agents identified under *in vitro* conditions were evaluated for their efficacy in managing leaf blight under field conditions. The lowest intensity was observed in vermi wash treated plots which was on par with application of *Trichoderma* in soil along with tuber treatment and spraying 0.2 % of TATA Samarth which also recorded highest yield.

Evaluation of a promising plant growth-promoting mycorrhizal fungus (PGPF)

A promising plant growth-promoting mycorrhizal fungus (PGPF) *Piriformospora indica,* a basidio-mycetous endophyte was evaluated for taro blight management. Pot culture evaluation on Taro cultivar Muktakeshi (Leaf blight resistant) and Sree kiran (Highly susceptible) varieties showed the fungal colonisation under light and confocal microscopes. Amplification of *P. indica* by species specific primer also confirmed the colonisation (Fig. 13a, b,c). *P. indica* was found to be an efficient growth promoter in both taro varieties tested and three weeks after planting the plants showed profuse growth and increased vigour compared to control. The root system showed significant growth in both the varieties tested (Fig. 14).



(a) (b) (c)

Fig. 13 a) Colonization of *P. indica* in roots of taro cultivar.
b) Confocal microscopy of colonization of *P. indica* in roots of taro cultivar. c) Amplification of *P. indica* by species specific primer

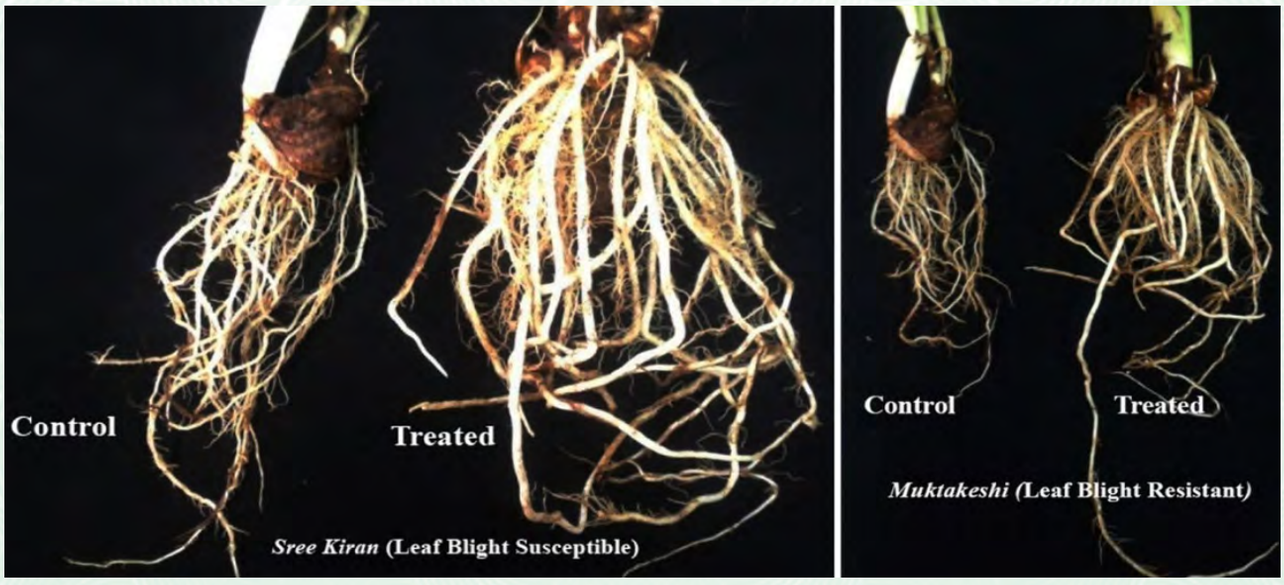


Fig. 14 Plant growth promoting activity of *P. indica* on taro cultivars

ICAR- ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

Principal Investigator:
Dr. (Mrs.) Amrita Banerjee

Co-Investigator:
Dr. G T Behere

A. DISEASE MANAGEMENT

Field evaluation of *Trichoderma* formulations

Talc based formulations of three *Trichoderma* isolates namely *Trichoderma harzianum, Trichoderma bravicompactum* and NRC-FBA-44 were prepared and evaluated under field conditions. The citrus trees with combined infection of citrus gummosis, citrus greening and citrus tristeza virus in the citrus orchard of Horticulture farm, ICAR Research Complex for NEH Region, Umiam were treated with formulated *Trichoderma* isolates. Four months after application of *Trichoderma* formulations, rhizospheric soils of treated citrus trees showed promising increase in *Trichoderma* population compared to control.

RUBBER RESEARCH INSTITUTE OF INDIA, KOTTAYAM

Principal Investigator:
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Co-Investigator:
Dr. T Saha

GENERATION OF MAPPING POPULATION

A mapping population of an interspecific cross between *Hevea brasiliensis* (clone RRII 105) and *H. benthamiana* (clone F4542) showed varying levels of resistance to *Phytophthora* spp. Eighty six progeny population derived from this interspecific cross was used for this study in an effort to construct a genetic linkage map for understanding genetic architecture of quantitative trait loci controlling *Phytophthora* disease resistance.

Phenotyping the mapping population for disease resistance

Leaf disc assay was used for screening the progeny population for their disease resistance potential. Lesion size produced following challenge inoculation with zoospore suspension on the leaf discs was measured periodically from 72-144 hours to assess disease reaction. The progeny populations were grouped into six categories based on the size of the lesion produced as A - highly resistant (0.0 – 0.8 cm), B - resistant (0.8 – 1.2 cm), C - moderately resistant (1.2 – 1.8 cm), D - moderately susceptible (1.8 – 2.2 cm), E - susceptible (2.2 – 2.8 cm), F – highly susceptible (2.8 – 3.5 cm). Segregation pattern for disease resistance trait in response to *Phytophthora* infection was assessed among 84 progeny population derived from the interspecific cross. Frequency distribution of disease resistance among the progeny was continuous, implying that *Phytophthora* disease resistance is a quantitative trait (Fig. 15). It is assumed that favourable alleles of multiple genes conferring resistance to *Phytophthora* are controlled by both parents, which has resulted in extreme phenotypes among hybrid progenies.

Genotyping the mapping population

Genotyping the parents of the mapping population (*H. brasiliensis* - clone RRII 105 and *H. benthamiana* - clone F4542) along with their progeny population of 86 individuals was carried out using co-dominant SNP markers and dominant SilicoDArT markers derived from DArT sequencing. A total of 24004 markers generated through DArT sequencing were used for the construction of linkage map. Eighteen linkage groups were observed with an average of about 1500 markers within each linkage group.

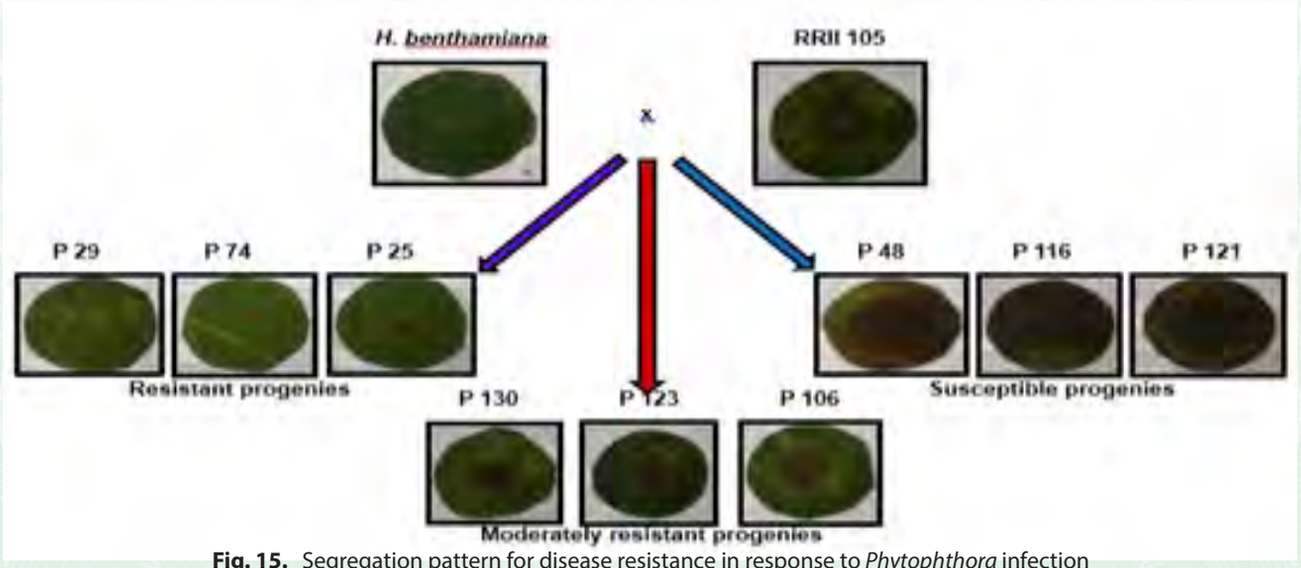

Fig. 15. Segregation pattern for disease resistance in response to *Phytophthora* infection

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

Principal Investigator:
Dr. I M Sharma

Co-investigator:
Dr. Usha Sharma, Dr. Kishore Khosla
Dr. Manju Modgil, Dr. D P Bhandari

A. DIVERSITY

Survey and incidence of collar rot disease in apple

Incidence of collar rot disease in the districts of Chamba, Sirmour and Kinnaur varied between 3.1-23.1, 1.6-26.1 and 0.7-12.6 per cent, respectively. Maximum incidence of this disease was observed in apple orchards in Jattota and Daand of District Chamba, followed by Dibber in Sirmour and Lippa in Kinnaur districts of Himachal Pradesh. Four isolates of *P. cactorum* were isolated from the soil/ diseased bark samples collected from surveyed areas. Isolates were classified based on their relative virulence and two were designated as highly virulent.

B. EPIDEMIOLOGY

Studies on host-pathogen-environment interaction for developing forecasting module

a. Disease progress studies

Under nursery conditions collar rot first appeared in the second week of April reached its maximum by the last week of August 2015, whereas in orchards disease was first observed on 5 April 2015 and reached maximum by the last week of October 2015. Disease progression was positively correlated with rainfall, soil moisture and minimum soil temperature and was negatively correlated with maximum soil temperature (Fig.16a, 16 b).

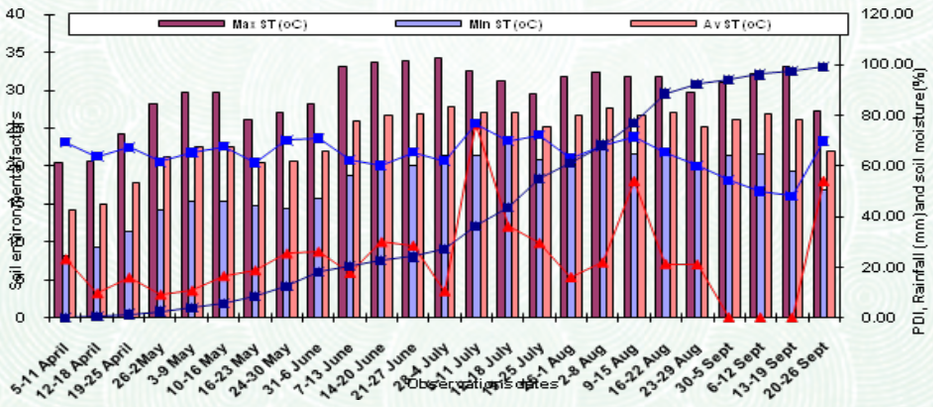

Fig. 16 a. Effect of soil environment at 10 cm depth on the development of collar rot in apple under nursery conditions

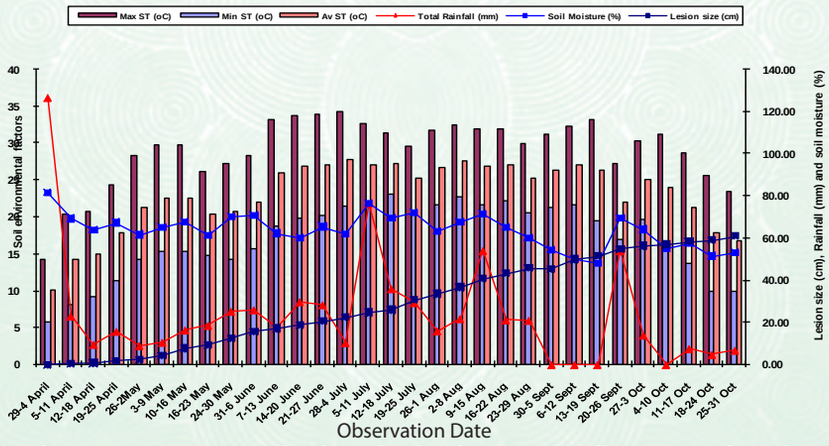

Fig. 16 b. Effect of soil environment at 10 cm depth on the development of collar rot in apple under orchard conditions

C. HOST RESISTANCE

Evaluation of seedlings of pollinizer cultivars of apple for resistance

Among the 30 pollinizer varieties, *Malus floribunda* showed maximum tolerance to collar rot disease under pot (11.4 per cent) and nursery 9.2 (per cent) conditions, followed by Stark Spur Golden , Bray Burn , Star Crimson, Winter Delicious , Gloster and Golden Delicious. Adaptive trials using grafted plants on resistant rootstocks laid at six hot spot locations showed that all apple rootstocks M9, M7, MM111were found to be the resistant to the disease.

D. DISEASE MANAGEMENT

Evaluation of molecules against collar rot disease

Out of the five molecules tested, Fosetyl Al and silicic acid (0.05%) sprays during April, June and August had reduced the disease severity to the extent of 83.2 and 89.4 per cent, respectively under nursery conditions .

Development of integrated disease management (IDM) strategy

Evaluation of different effective management inputs under pot conditions indicated that pre-inoculation treatments with BCAs, fungicides and amendments reduced disease incidence when compared to post-inoculation applications. Amongst different treatments, combined applications of BCAs (TH5/ THM2 + BS11/BS4/ KB6) and fungicides resulted in 94 per cent disease control when applied 7 days prior to inoculation.

Under nursery conditions combination of BCAs (THM2/TH5+BS11/BS4) or amendments (mustard cakes + murraya leaf) or BCAs + mustard cake with Curzate or Cabrio Top were found to be effective and resulted in 98 per cent control of disease when applied 20 days prior to planting of seedlings.

Under orchard conditions, the addition of cow urine decoction (CUD) thrice @7.5% (10L/tree) during March, June and August accompanied with approach grafting (AG) and two applications of Curzate (0.3%) during April and August was found to be most in decreasing the severity of collar rot and for growth promotion. In an another trial, results indicated that combined application red soil +cow urine decoction +approach grafting was highly effective in increasing the shoot length of plants as well as lesion recovery at the collar portion (38.4 per cent) .

ICAR-INDIAN INSTITUTE OF HORTICULTURAL RESEARCH, BENGALURU

Principal Investigator:
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Co-investigator:
Dr. A K Saxena

A. DIVERSITY

Six new isolates of *Phytophthora nicotianae* collected from Hosur and Dharmapuri (Tamil Nadu), Doddaballapur, Rajankunte, Hessaraghatta (Karnataka) were added to the culture collection having two isolates of *Phytophthora nicotianae* from crossandra maintained at IIHR, Bengaluru. The pathogens were identified as *P. nicotianae* by morphological and molecular tools (ITS sequence amplification). Five new isolates of *P. nicotianae* (four from Kodagu and one from Sikkim) infecting passion fruit have been added to the culture collection. Besides crossandra and passion fruit, *Phytophthora nicotianae* from anthurium and pomelo and *P. palmivora* from papaya were also isolated and added to the culture collection.

Fig. 17 Wilt reaction in local variety of crossandra, 1. control; 2 - 4: inoculated with *P. nicotianae*)

Fig. 18 Wilt in reaction in Arka Kanaka
1. Control; 2 and 3 inoculated with *P. nicotianae*)

B. HOST RESISTANCE

Screening for disease resistance

Five varieties *viz.,* Arka Shravya, Arka Shreya, Arka Kanaka, Arka Ambara and local variety were tested for disease resistance by artificially inoculating with *P. nicotianae*. The local variety showed highest mortality (66.66 per cent), followed by 22.22 per cent in Arka Kanaka, and 11.11 per cent in Arka Shravya and these varieties were grouped as susceptible genotypes (Fig. 17 and 18).

C. MANAGEMENT

Evaluation of fungicides and bioagents for the management of crossandra wilt and passion fruit

Fungicides *viz.* copper oxychloride, Fosetyl Al, fenamidone + mancozeb, metalaxyl and mancozeb were tested *in vitro* at different concentrations (0.5, 0.1, 0.15 and 0.2 per cent) and the molecules, fenamidone + mancozeb, metalaxyl and mancozeb were found very effective. The fenamidone + mancozeb tolerant isolate of *Trichoderma harzianum* GJ16B was tested for its efficacy against *P. nicotianae* affecting crossandra wilt under pot culture conditions.

ICAR-INDIAN INSTITUTE OF SPICES RESEARCH, KOZHIKODE

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Co-Investigator:
Dr. K Nirmal Babu, Dr. Johnson K George
Dr. R Suseela Bhai, Dr. Santhosh J Eapen
Dr. R Praveena, Mrs. P. Umadevi

A. DIVERSITY

New *Phythophthora* isolates of black pepper and cocoa collected from Kozhikode District and four American Type Culture Collections (ATCC) of *Phytophthora tropicalis, P. capsici, P. palmivora* and *P. meadii* procured were revived and maintained in the National Repository of *Phytophthora*. Presently 440 isolates of *Phytophthora* including ATCC isolates are being maintained in the repository. The morphological and physiological characters of ATCC cultures *P. tropicalis, P. meadii* and *P. capsici* were studied and compared with black pepper isolates.

ITS phylogeny and MLST analysis of capsici/tropicalis groups indicated the presence of two separate subclades within Clade 2, deviating from *P. capsici* and *P. tropicalis*. Morphological studies of capsici/tropicalis groups showed the presence of mixed characters that are deviant from the type description. Preliminary studies based on SSCP analysis also indicated the existence of inter-species hybrids among *Phytophthora* isolates of black pepper.

Characterization of *Phytophthora* sp. infecting nutmeg: Eight *Phytophthora* cultures (13-01 to 13-06, 13-55 and 98-68) were characterized based on morphological, physiological and molecular characters. Majority of the isolates belonged to A1 mating type except 13-06 (A2 mating type). Blast analysis of ITS rDNA sequences, restriction analysis using MSP1 and MLST analysis with mitochondrial and nuclear genes revealed that nutmeg *Phytophthora* isolates were grouped into a separate clade closely related to *P. meadii*. Further through SSCP analysis it was confirmed that, nutmeg isolates were entirely different from *P. meadii* isolates of cardamom and cocoa but closer to coconut *P. meadii*. Based on the morphological, physiological and molecular characterization, it is hypothesized that the nutmeg isolates could be a hybrid with parentage, *P .citrophthora* x *P.meadii* or *P. capsici* or *P. tropicalis*.

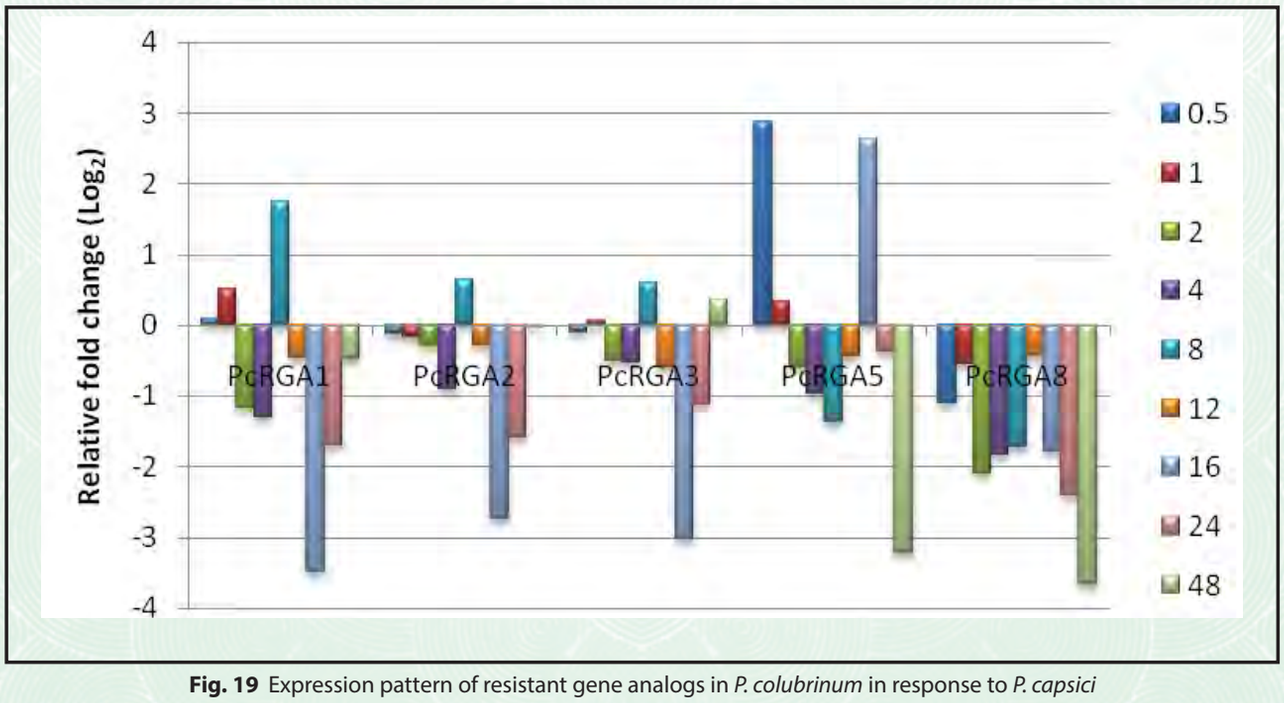

Fig. 19 Expression pattern of resistant gene analogs in *P. colubrinum* in response to *P. capsici*

New isolates of burrowing nematodes: Nine different isolates of *Radopholus similis* were collected from black pepper (7 isolates) and banana (2 isolates) from the surveys conducted in Thiruvananthapuram, Thrissur, Malappuram, Idukki and Wayanad districts of Kerala.

Identification of marker genes in *Radopholus similis:* For characterization of *R. similis* population from India, different marker genes *viz.,* mitochondrial cytochrome c oxidase gene (cox1), NADH dehydrogenase gene (nd1), the internal transcribed spacer region (ITS1-5.8S-ITS2) of the rRNA gene, D3 expansion domain segments of the 28S rRNA gene, 18S ribosomal RNA gene and 28S ribosomal RNA gene were amplified, cloned, sequenced using specific primers. The sequences obtained were submitted to NCBI (Acc. Nos. KU530150 - KU530152).

B. DIAGNOSTICS

The real time PCR based method developed for the detection and quantification of *R. similis* in soil was further used for detecting the presence of *R. similis* in soil by using soil DNA isolated from *R. similis* infected (positive) and sterile (negative) soil sample. Only the positive samples showed amplification.

C. HOST PLANT RESISTANCE

Identification of R genes in *Piper colubrinum:* Around 1289 candidate R gene homologues were mined from *P. colubrinum* transcriptome by reverse alignment using amino acid sequences of 42 known R genes. The sequences were functionally annotated and clustered to 91 clusters by h-cd-hit on CD-HIT suite using multiple CD-HIT runs.

Differential expression analysis of Resistance Gene Analogs

The expression pattern variability of NBS LRR *P. colubrinum* RGAs (PcRGAs) in *P. colubrinum* was analyzed (Fig. 19). Basal level expressions of NBS-LRR PcRGAs were observed in *P. colubrinum*. In challenge inoculated *P. colubrinum* leaves, there is a marginally high expression of PcRGA1 (at 0.5 and 1 hpi), PcRGA2 (at 8 hpi), PcRGA3 (at 1, 8 and 48 hpi) compared to uninoculated control. In the case of PcRGA1, maximum fold change recorded was at 8 hpi (3.36) and after 8 hpi expression of PcRGA1 is downregulated. Significantupregulation of PcRGA5 was observed at 0.5 hpi (7.36 fold) which was downregulated after 24 hpi, while the expression of PcRGA8 was downregulated at all time points post inoculation.

The expression of NBS LRR *Piper nigrum* RGAs in *P. colubrinum* and vice versa was analyzed by relative quantification of mRNA transcripts with mock inoculated control (Fig. 20). While comparing expression of PnRGAs in IISR Shakthi, 04-P24-1, Subhakara and *P. colubrinum,* PnRGA1 and PnRGA24 were identified as potential candidate genes. Expression of PnRGA24 is significantly higher in *P. colubrinum,* IISR Shakthi and

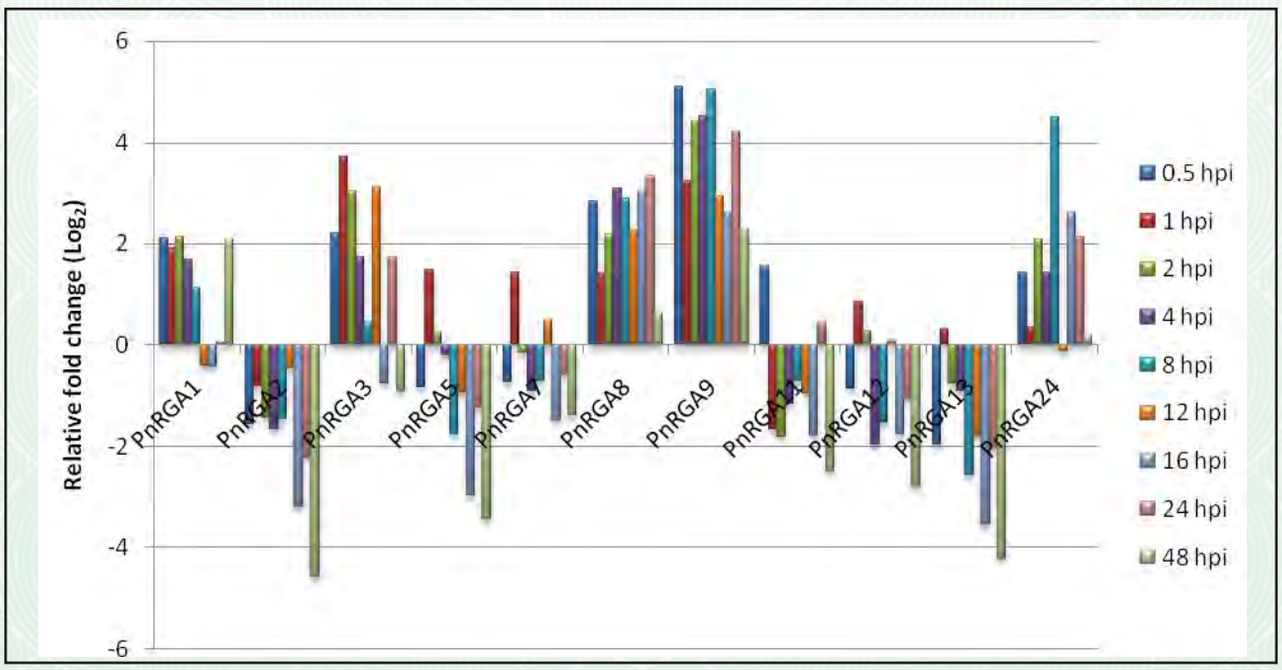

Fig. 20 Expression pattern of PnRGAs in *P. colubrinum*

04-P24-1 compared to susceptible variety Subhakara, with the maximum being in *P. colubrinum*. Expression of PnRGA1 was significantly higher in IISR Shakthi and *P. colubrinum*. A low level of PnRGAs observed under unchallenged condition suggests the constitutive ability of the plant R-genes for pathogen surveillance, pathogen perception and induce downstream defense responses. However, the expression of PnRGAs was found to be expressed during the early hours of infection and down regulated towards the latter phases of infection. The PnRGAs which are not significantly expressed in *P. nigrum* (PnRGA5, PnRGA7, and PnRGA8) were highly expressed in *P. colubrinum*. However, gene silencing studies and genetic transformation studies are required to confirm the functionality of R gene.

D. HOST PATHOGEN INTERACTION

Expression analysis of defense-related proteins in *Piper colubrinum*

Real time PCR analysis was done for defense-related genes *viz.,* peroxidase, lipoxygenase, superoxide dismutase, chalcone synthase, chalcone isomerase, catechol oxidase, phenyalanine ammonia lyase (PAL), catalase, cinnamoyl coA reductase, polyphenol oxidase, EDS1, serine threonine kinase, chitinase II, senescence associated protein, allene oxide synthase and PR proteins like PR-1 and PR-14 in *Piper colubrinum* challenge inoculated with *Phytophthora capsici* (Fig. 21). Expression of peroxidase (450 folds)

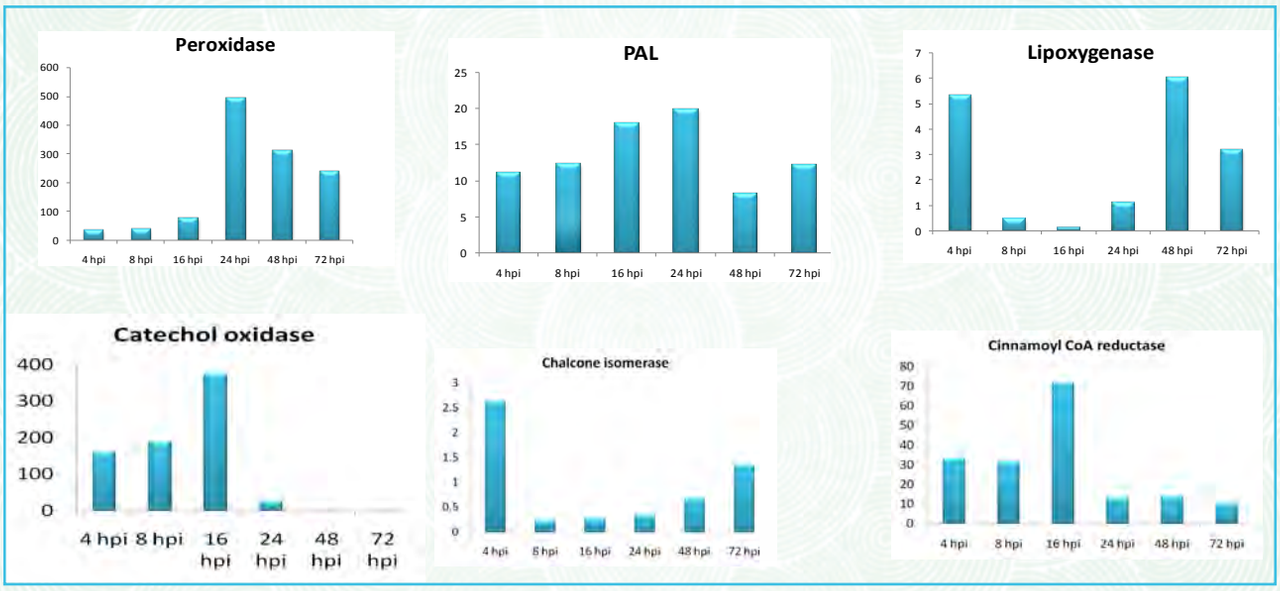

Fig. 21 Expression of defense-related genes in black pepper inoculated with *Phytophthora capsici.*

Fifty seven genotypes of black pepper along with *Phytophthora* resistant *P. colubrinum,* tolerant lines *viz,* IISR Shakthi, IISR Thevam and P24-04 and susceptible lines - Sreekara and Subhakara were screened for *Phytophthora* resistance using 25 ISSR markers and 15 SSR markers. The dendrogram constructed based on Dice similarity coefficient divided the population into three major clusters. The first cluster was formed by *P. colubrinum* which stood distinct with 32 percent similarity with rest of the black pepper clusters. The other two major clusters which comprised black pepper genotypes shared 80% similarity. The second and third clusters were further divided into two sub-clusters each. The similarity coefficient ranged from 0.32 to 0.99. All the genotypes were separated. The *Phytophthora* tolerant genotypes found a place in the second cluster .

and PAL (18 folds) was significantly greater at 24 hpi. The genes like catechol oxidase (350 folds), cinnamoyl coA reductase (70 folds) and polyphenol oxidase (220 folds) were found to be expressed maximum at 16 hpi. Lipoxygenase (5.4 folds), chalcone isomerase (2.5 folds), EDS1 (1.85 folds) showed increased expression at early stages of inoculation *i.e.* at 4 hpi. Genes like super oxide dismutase and catalase were also found to be expressed during early stages of interaction at 4 hpi (5.03 and 4.89 folds, respectively). The PR protein gene PR-1 showed extremely high level of expression about 490 folds at 16 hpi and PR-14 was showing maximum 10 folds of expression at 4 hpi. The gene allene oxide synthase didn't show much increase in expression except (1.3 folds) at 24 hpi and serine threonine kinase showed maximum expression at 72 hpi (1.63 folds). Down regulation of chitinase II and senescence

associated protein genes was found compared to un-inoculated control.

Global proteomics of defense mechanism in black pepper: Label free proteomics strategy was applied to bring out the protein expression abundance and post translational modifications (PTMs) on tolerant (IISR Shakthi) and susceptible (Subhakara) genotypes. The leaf proteins were extracted from plants inoculated with *P. capsici* at 12 and 24 hpi along with control leaf. LTQ-Orbitrap Discoverer platform was used to fingerprint the quantitative expression of proteins during pathogen infection. The peptide peak data obtained was then annotated and relative expression of peptides was analyzed using Hi-3 (Average normalized abundance). In total, 299 proteins were analyzed out of which 84 proteins were found to have above 4 fold to 973 fold increase in expression and 38 of them were found to be upregulated at 24 hpi. In tolerant genotype the pathogen was suppressed by pattern triggered immunity (PTI) which was triggered by receptor like kinases RLKs, RPP13 (R gene). These were identified as important pattern recognition receptor against *Phytophthora* in black pepper. Salycylic acid (SA) mediated SAR was identified during pathogen infection. PR protein (with antifungal activity) production was found to be more in tolerant black pepper. In susceptible, PTI was found to be weak with alteration in host physiology. Effector triggered immunity (ETI) by *Phytophthora* effector proteins and PRM1 (R gene) with jasmonic acid mediated suppression of SA. Production of PR5 (Thaumatin) protein and PAL production signified the susceptibility. The peptide data was then integrated with the Piper transcriptome DB on IISR Shakthi Illumina GLX 2X platform, annotated with Blast2Go and the R gene families in IISR Shakthi were grouped.

E. MANAGEMENT

Effect of secondary metabolites of endophytic fungi on *P. capsici:* The secondary metabolites extracted from nine different endophytic fungi were tested against *P. capsici*. The maximum mycelial inhibition (68 per cent) was recorded by the isolates BPEF11 (*Diaporthe* sp.), BPEF83 (*Phomopsis* sp.), BPEF41 *(Annulohypoxylon niten),* BPEF25 and BPEF38 *(Daldinia eschscholtzii)* and the rest of the four isolates showed more than 55 per cent inhibition of *P. capsici* under *in vitro* conditions. The metabolites also inhibited production of *Phytophthora* sporangia at 25°C for 48 h under light. When ethyl acetate extracts of fungal metabolites dissolved in dimethylsulfoxide were tested using *Phytophthora* inoculated cut shoots of black pepper, metabolites of seven out of nine isolates (BPEF11, BPEF25, BPEF41, BPEF72, BPEF73, BPEF81, and BPEF83) showed 90 per cent lesion inhibition.

Evaluation of actinomycetes consortia: Combined application of IISR Act 2 *(Ketosatospora setae)* + IISR Act 5 (*Streptomyces* sp.) and IISR Act 2 + IISR Act 9 (*Streptomyces tauricus*) was found effective in reducing the soil nematode population to an extent of 58-75%. Based on this observation, a new pot culture experiment was initiated to study the effect of these consortia on combined infection by *Phytophthora* and nematodes in black pepper rooted cuttings.

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A. DISEASE MANAGEMENT

Induced resistance against *Phytophthora* seedling blight in castor by *Trichoderma*

Further studies on induced resistance against *Phytophthora* seedling blight in castor by *Trichoderma harzianum* Th4d have been taken up to identify the elicitor molecules having role in inducing defense in castor. Metabolites secreted during castor-*Trichoderma* interaction in hydroponic culture have been characterized by LC-MS and identified anthraquinone, harzianopyridone, ellagic acid and taurene. HPLC and GC analysis showed production of amino acids like amino butyric acid (cystathionine, cysteine, lysine), fatty acids (dichloroacetic acid, dodecyl ester) and organic acids (benzoic acid, silane, (dodecyloxy) trimethyl- (CAS), and 1,2-benzenedicarboxylic acid). Many of these compounds known to have role plant growth promotion and some act as antimicrobial agents.

Screening of bioagents against *Phytophthora nicotianae* in castor

Greenhouse tests

In greenhouse trials with castor cultivar GCH 4, seed germination was found to be significantly high in bioagent treatments when compared to the pathogen check . Among all the bio agents seed treatments, *T. harzianum* Th4d and *T. harzianum* ThN2 showed least disease severity of *Phytophthora* leaf blight (15.0%) followed by *P. fluorescens* Pf3 with (20.0%) disease severity whereas pathogen check recorded 60% disease severity. The study clearly showed induction of defense in castor against *Phytophthora* seedling blight.

B. HOST RESISTANCE

Reaction of safflower cultivars to *Phytophthora nicotianae*

Total 31 safflower genotypes were screened against *Phytophthora nicotianae* leaf blight and among them two genotypes DSI-103 & GMU-3263 were resistant. Two genotypes (DSI-104 & EC-523368-2) were moderately resistant with less than 20% disease severity, genotypes *viz*. SSF-GMU-6878, GMU-1205-2, SSF-1302, GMU-1946, GMU-4912, SSF-658 and Manjeera recorded less than 30% severity of *Phytophthora* leaf blight.

Fusarium

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A. DIVERSITY

Cytochrome c oxidase I (COX1) and RNA Polymerse II (RPB 2) sequence analysis

Seventy one isolates of *Fusarium oxysporum* f. sp. *ciceris* (Foc) were amplified by using a set of primers *viz.,* COX 1F and COX 1R which produced ≈500 bp fragment in all the isolates of the pathogen. These isolates were amplified RPB 2 primers, namely, RPB2 5F and RPB2 7cR which produced ≈1200 bp fragment in all the isolates of the pathogen. These isolates were representative of 14 different states and 5 pulse growing agro ecological zones *viz.,* North Eastern Plane Zones (NEPZ), North Western Plane Zones (NWPZ), North Hill Zones (NHZ), South Zones (SZ) and Central Zones (CZ) of India. These isolates were representing 8 races of the pathogen.

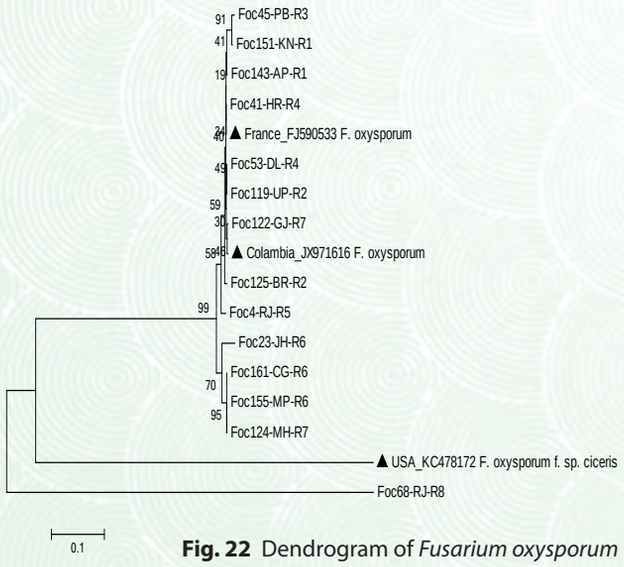

Fig. 22 Dendrogram of *Fusarium oxysporum* f. sp. *ciceris* isolates for COX 1 gene

The cox-1 and RPB2 analysis were not found to be suitable markers for grouping of *F. oxysporum* f. sp. *ciceris* (Foc) races representing different geographical regions of India as well as places of origin. The cox-1 partially grouped the isolates into different race specific clusters. The cox-1 distinguished the races of isolates into seven groups. The isolates belonging to the race 4 and race 6 were only differentiated in clustering but other isolates belonging to different races were not differentiated properly (Fig. 22). The RPB2 did not group the isolates into different race specific clusters. It has distinguished isolates of races into seven groups but none of isolates belonging same races were clustered together (Fig. 23).

B. HOST PATHOGEN INTERCATION

Differential expression pattern of MAP kinase gene family

Conventional and real time PCR assays were used to determine differential expression pattern in the mitogen-activated protein (MAP) kinase gene family of Foc infected resistant (GPF 2) and susceptible (JG 62) chickpea plants. Chickpea seedlings at the

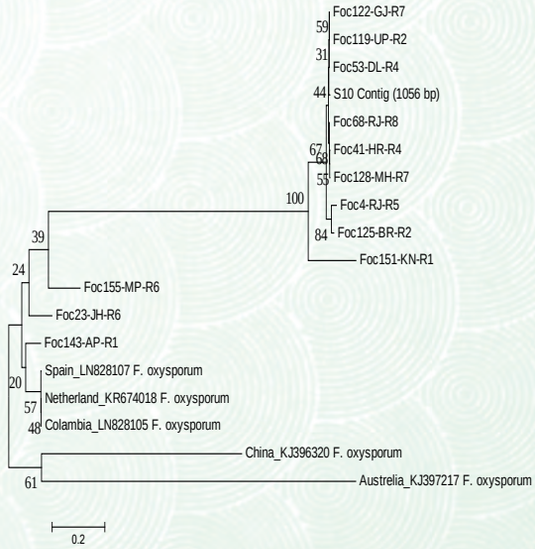

Fig. 23 Dendrogram of *Fusarium oxysporum* f. sp. *ciceris* isolates for RPB2 gene

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 results of the expression analysis of MAP genes at 1, 2, 3 and 4 days after inoculation (dai) between treated and untreated susceptible (JG 62) and resistant (GPF 2) cultivars of chickpea performed by using conventional

PCR showed the presence of expected amplicon size for MAP genes, namely, MAPK- F1R1 (248 bp) and MAPKKK- F2R2 (196 bp).

Real-time PCR clearly showed that MAP kinase (MAPK) gene was differentially expressed at different time intervals in both treated *(T. harzianum)* and untreated plant samples. The expression analysis indicated that Foc infection up-regulated MAPK gene in susceptible variety, whereas in resistant variety gene is down-regulated as compared to control (Un-inoculated). The treatment of *T. harzianum* + Foc also showed gene up-regulation in susceptible variety but in case of resistant cultivar gene was down regulated except 1 day after inoculation (dai) as compared to control. Considering all the treatments with Foc and *T. harzianum,* the MAPK gene was up-regulated in susceptible variety whereas, it was down-regulated in resistant variety. The expression was the highest in Foc treated plants followed by *T. harzianum* + Foc and *T. harzianum* in the susceptible variety whereas, gene of resistant variety showed highest down expression in Foc treated plants followed by *T. harzianum* and *T. harzianum* + Foc. The different treatments of chemicals also indicated that MAPK gene was up-regulated in susceptible as well as resistant variety as compared to un-inoculated control. The treatment salicylic acid + Foc showed highest expression of the gene in susceptible and resistant variety compared to control (Fig. 24 a & b).

The MAP kinase kinase kinase (MAPKKK) gene was also differentially expressed at different time intervals in both treated and untreated plant samples. The gene was up-regulated with treatment of *T. harzianum* + Foc at 2 dai followed by 3 dai and it was down regulated 1 dai and 4 dai, whereas in the case of Foc inoculated sample gene was up-regulated except 1 dai as compared to the control in susceptible variety. The MAPKKK gene of resistant variety showed up-regulation with the treatment of *T. harzianum* + Foc and the highest expression found at 3 dai, whereas Foc inoculated sample was up-regulated at 1 dai and 3 dai, but it was down-regulated in 2 dai and 4 dai against control . The expression was highest in *T. harzianum* + Foc treated plants sample followed by Foc and T. harzianum in the susceptible as well as resistant variety. The plants treated with different chemicals indicate that MAPKKK gene was up-regulated in susceptible as well as resistant variety as compared to un-inoculated control. The average expression pattern of MAPK and MAPKKK genes were similar in both varieties against different chemical treatments (Fig. 25 a and b).

C. DISEASE MANAGEMENT

Fifteen isolates of *Trichoderma* which were found to be promising under PhytoFuRa project were evaluated at field level for chickpea seed treatment formulation. The results indicated that Isolate T-1 (132) was superior for promotion of seed germination and reducing wilt

Table1: Evaluation of PhytoFuRa isolates of *Trichoderma* species on chickpea growth promotions and reduction of wilt incidence under field conditions after 90 DAS

Treatment	Total number of plants germinated	Wilt incidence (%)	Root length (cm)	Shoot length (cm)	Total biomass (g)	No. of branches	No. of nodules	Dry weight of shoot (g)	Dry weight (g)	Yield (g)
T1	132	32.4	9.75	39.25	30.00	4.50	2.50	14.00	4.50	72.50
T2	121.5	41.6	11.25	40.75	40.00	4.00	13.75	10.00	7.00	67.50
T3	129.5	43.5	9.38	41.50	35.50	3.25	9.00	18.75	5.50	77.50
T4	120	39.9	9.25	40.00	38.00	4.75	15.25	15.50	4.25	122.50
T5	119	39.5	10.00	40.75	52.50	5.75	11.75	23.75	7.00	97.50
T6	118	39.8	9.63	43.00	42.50	3.75	8.00	18.75	6.50	102.50
T7	125	45.6	8.25	42.50	28.00	4.00	1.50	12.75	2.75	127.50
T8	112.5	36.4	9.25	38.50	28.00	3.50	5.50	15.00	3.00	92.50
T9	127.5	41.1	10.25	38.25	22.50	3.00	4.25	16.25	4.25	55.00
T10	125.5	44.0	9.25	40.50	28.75	3.50	1.75	13.75	1.75	145.00
T11	114.5	36.0	9.25	38.50	31.75	3.75	7.75	18.00	6.00	122.50
T12	113	36.5	11.25	41.50	50.00	4.50	6.75	32.50	8.50	75.00
T13	118	42.4	12.00	39.00	20.00	4.50	6.25	18.75	3.00	97.50
T14	121.5	41.2	9.75	40.50	42.50	4.75	9.50	19.25	3.25	82.50
T15	129	38.8	8.50	34.75	30.00	3.50	8.50	32.50	2.00	97.50
Mock	130	37.6	11.00	43.25	35.00	4.00	10.50	17.50	4.50	95.00

incidence by 32.4% followed by isolate T-8 (36.4%). Among all isolates of PhytoFuRa the highest total biomass production was observed in T-5 (52.50 g) followed by T-12 (50 g) isolate. Total yield was observed for the isolate T-10 (145 g) followed by T-7 (127.50g) and T-4 and T-11 (122.50g) (Table 1).

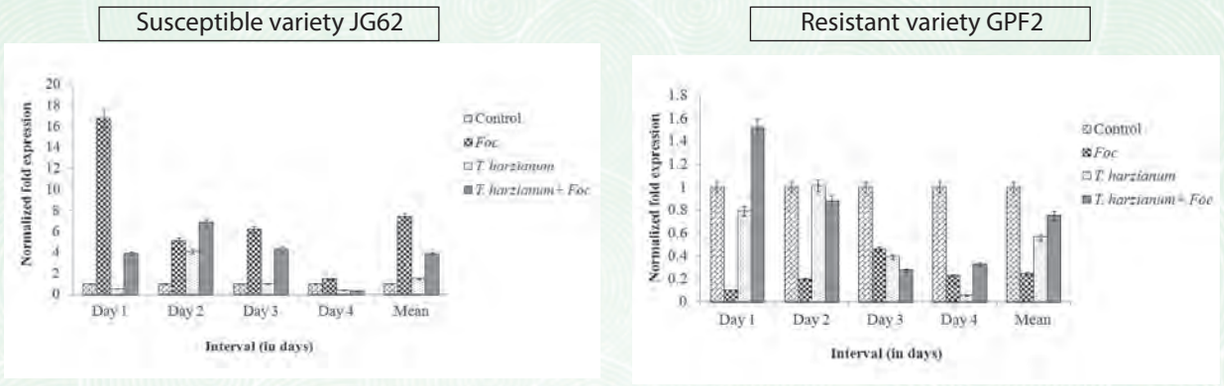

Fig. 24 a Expression profiling of MAPK gene in chickpea by using primer MAPK- F1R1

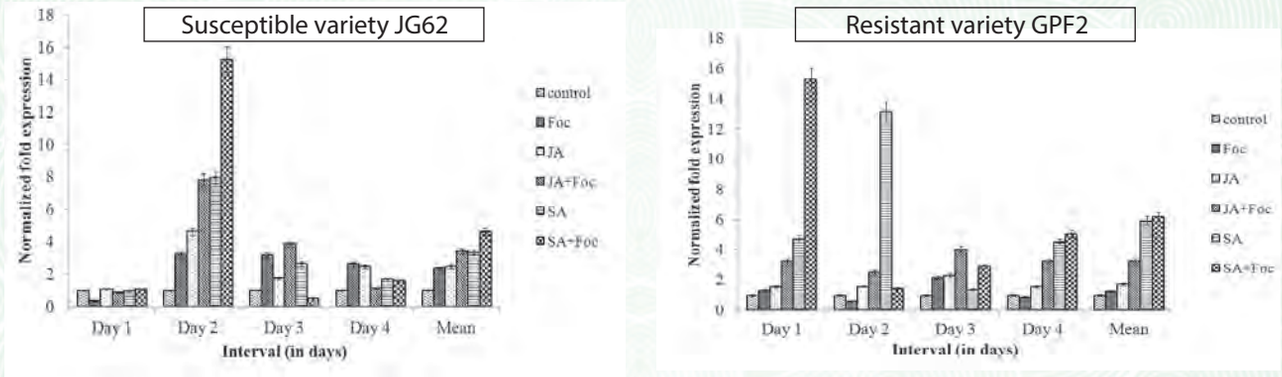

Fig. 24b Expression profiling of MAPK gene in chickpea with the primer MAPK- F1R1 with different chemical treatment

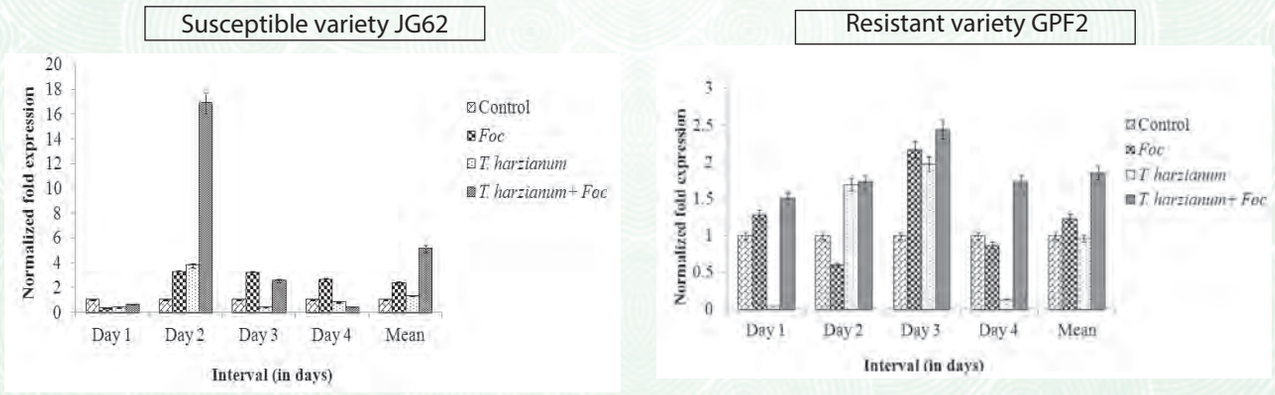

Fig. 25a Expression profiling of MAP KKK gene in chickpea with the primer MAP KKK- F2R2

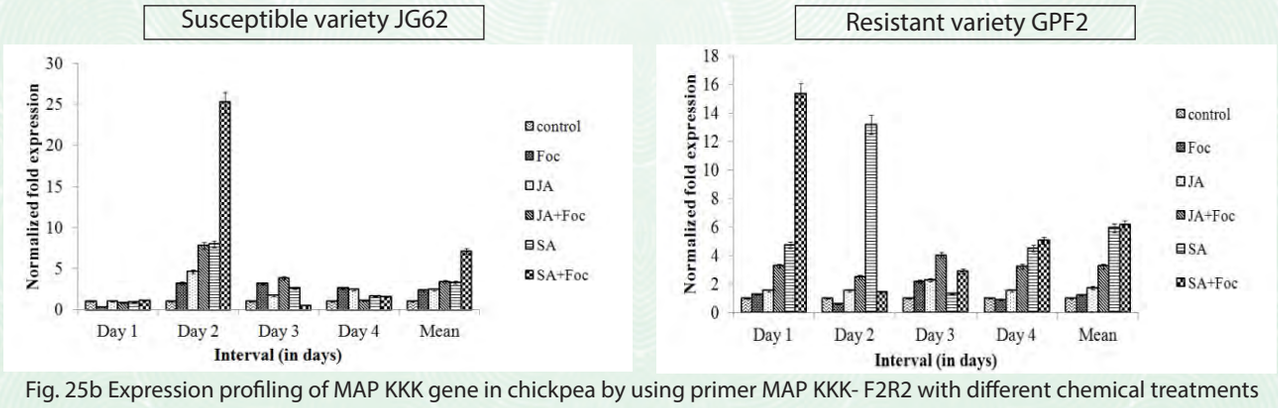

Fig. 25b Expression profiling of MAP KKK gene in chickpea by using primer MAP KKK- F2R2 with different chemical treatments

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A. DIVERSITY

Six new isolates of *F. oxysporum* f. sp. *gladioli* (FOG) were collected from Shillong, Pune and IIHR field (Fig. 26) and four isolates of *F. oxysporum* f. sp. *dianthi* (FOD) were collected from IIHR, TNAU and Karnataka . Morphological identification of isolates was done based on mycelial and spore characteristics, production of chlamydospore and pigmentation on PDA plates. Molecular characterization was done by polymerase chain reaction with ITS F/R and TEF F/R primers. FOG/FOD cultures mass multiplied on sorghum grains were mixed with sand and inoculated to carnation plants/ gladiolus corms in pots 15 days after planting in sterile soil. Three types of carnation varieties were used for the screening namely Arka Flame, Arka Tejus and H13. Arka Flame and Arka Tejus were found to be resistant to *Fusarium* wilt whereas, Gladiolus variety white prosperity was showing susceptibility to *Fusarium* wilt. Isolates of *F. oxysporum* f. sp. *gladioli* were proved as virulent isolates.

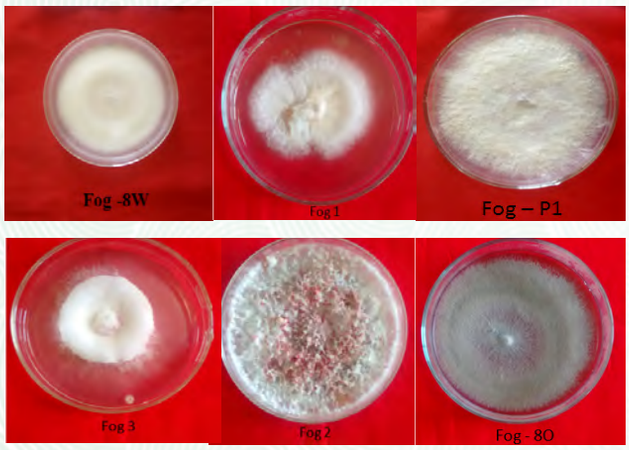

Fig. 26 *Fusarium* isolates from gladiolus

B. HOST PLANT RESISTANCE

Fusaric acid crude extracts were isolated from FOD solan, FOD IIHR7, FOD IARI C4 and FOG solan isolates. Extracted crude form of fusaric acid was used for screening of gladiolus and carnation varieties. The extracts were directly injected into the root established carnation plants with the help of syringe needle, same way gladiolus corms were also treated with crude extracts of FA. Three IIHR varieties of carnation plants (Arka flame, Arka Tejus, and H13) and Gladiolus White prosperity were used for screening (Table 2, Fig. 27 a and b).

Table 2. Response of gladiolus and carnation to fusaric acid treatment.

Treatment	Concentration of FA used	Response observed	
		Carnation	Gladiolus
T1	Control (Sterile water)	No symptoms	No symptoms
T2	250µl (250µl+0µl sterile water)	No difference in dilution. All showing wilt symptoms in 5 days	Gradual increase on the symptom expression with increase in concentration observed in susceptible variety. 75µl was sufficient.
T3	100µl (100µl+150µl sterile water)		
T4	75µl (75µl+175µl sterile water)		
T5	50µl (50µl+200µl sterile water)		
T6	25µl (25µl+225µl sterile water)		

Fig. 27 a Response of gladiolus (variety white prosperity) to different doses of fusaric acid

Fig. 27 b Response of carnation (variety H13) to different doses of fusaric acid

C. GENOMICS

Molecular characterization of resistant gene analogs from carnation against *Fusarium oxysporum* f. sp *dianthi* with degenerative oligonucleotide primers for the identification of genes conferring resistance to *Fusarium* wilt in carnation is in progress. Three varieties namely Arka Flame, Arka Tejus and H13 are being used for the disease resistance screening .

D. DISEASE MANAGEMENT

A field trial was conducted to evaluate the efficacy of *Trichoderma harzianum* (carbendazim tolerant isolate GJ16B from NBAIR), carbendazim and captan for the management of *Fusarium* wilt in Gladiolus (Fig. 28 a & b). *Trichoderma* treatments and fungicide treatments were on par with their effect in wilt management. The combined treatment of *Trichoderma* and fungicides were found to be more effective (5.6% to 8.7% wilt incidence) than single application of chemicals alone (12.3%).

Fig. 28 a. Field trial for the management of gladiolus wilt (variety: white prosperity)

Fig. 28 b. Wilting of gladiolus plants in control plot

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A. HOST RESISTANCE

Resistant sources to *Fusarium* wilt in safflower

Total 31 safflower genotypes were screened against *Fusarium* wilt and among them two genotypes *viz.,* DSI-104 and GMU-3263 have recorded significantly low wilt incidence (10 & 15%) (Fig. 29). Ten genotypes (DSI-103, GMU-4912, SSF-658, Bhima, GMU-5133, SSF-GMU-6878, EC-523368-2, Manjeera & NARI-38) recorded less than 30% wilt incidence .

Fig. 29 Reaction of safflower cultivars to *Fusarium* wilt

B. GENOMICS

Six wild species have been repeatedly confirmed for high resistance against *Fusarium* wilt in wilt-sick pots in glasshouse (Fig. 30) .

Fig. 30. Resistance reaction of wild species against wilt in glasshouse

Marker-assisted selection for wilt resistance in interspecific derivatives

Nira x C. oxyacantha: Marker-assisted selection was practiced for wilt resistance using SSR markers flanked to wilt resistance in F2 to F6 generations of (Nira x *C. oxyacantha)* (Fig. 31). Simultaneously phenotyping for reaction against wilt was done by growing parents and various generations (F2 to F6) in wilt sick plot. Wilt incidence in various generations has ranged from 0-7%. It indicated successful transfer of wilt resistance from resistant wild species, *C. oxyacantha* into wilt susceptible cultivated variety 'Nira' belonging to cultivated species *(C. tinctorius)* (Fig. 32).

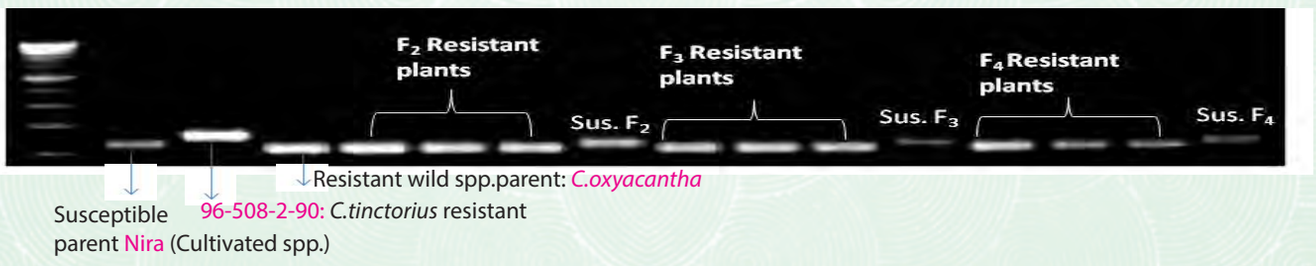

Fig. 31 Marker-assisted selection for wilt resistance using SSR marker in F2 to F4 generations of Cross (Nira x *C. oxyacantha*).

Fig. 32 Resistant reaction (R) of (Nira x. *C.oxyacantha*) and susceptible (S) reaction of 'Nira' in sick plot

Nira x C. palaestinus : Marker-assisted selection was practiced for wilt resistance using SSR markers flanked to wilt resistance in F2 to F5 generations of (Nira x *C. palaestinus*). Simultaneously phenotyping for reaction against wilt was done by growing parents and various generations (F2 to F5) in wilt sick plot. Wilt incidence in various generations has ranged from 0-7%. It indicated successful transfer of wilt resistance form resistant wild species, or C. palaestinus into wilt susceptible cultivated variety 'Nira' belonging to cultivated species *(C. tinctorius)*.

Identification of SSR markers flanked to wilt resistance in C. lanatus : BSA was done in F2 generations of crosses (Nira x *C. lanatus*). SSR markers flanked to wilt resistance in *C. lanatus* were identified. Markers flanked to wilt resistance were identified in both crosses. MAS was practiced in F2 and simultaneously F2 was screened against wilt in wilt sick plot. The markers were validated in F2.

C. DISEASE MANAGEMENT

Effect of fungicides and biological agents on *Fusarium* wilt in safflower under field conditions

The management trial using chemical fungicides, combination fungicides and biological control agents indicated that *T. harzianum* Th4d SC @ 2ml/kg and cymoxanil 8% + mancozeb 64% @ 0.2% treatments were most effective recording significantly low incidence of *Fusarium* wilt (17.6 & 18.2 per cent respectively) whereas control plots recorded a wilt incidence of 36.3 per cent (Fig.33).

Fig. 33 Effect of fungicides and biological agents on *Fusarium* wilt in safflower

Screening of bioagents against *Fusarium oxysporum* f.sp *ricini* in castor

A total of six bioagents [*T. harzianum* (three strains) and *P. fluorescens* (three strains)] were screened against *Fusarium oxysporum* f.sp. *ricini* using (Germination towel method). Seed treatment with bioagents showed increase in seed germination and vigour index. *T. harzianum* Th4d treatment gave highest vigour index compared to all other bioagents. Among all the treatments, *T. harzianum* N2, *T. harzianum* Th4d and *P. fluorescens* Pf 3 (20%) showed least wilt incidence and high reduction in wilt incidence (67.8%) followed by *T. harzianum* Th N1 and *P. fluorescens* Pf 2 (30%) when compared with pathogen check (90 per cent).

Greenhouse tests

In greenhouse trials with castor cultivar GCH 4, seed germination was found to be significantly high in bio agent treatments when compared to the pathogen check (Table 3). Among all the bio agents low wilt incidence was recorded with *P.fluorescens* Pf2 (30%) followed by *T. harzianum* Th N2 (45%) and *T. harzianum* Th4d (55%).

Table 3. Efficacy of bio control agents in controlling wilt of castor

Treatment	Germination (%)	Wilt incidence (%)	Reduction over pathogen check (%)
T.harzianum Th4d	100.0 (90.0)	55.0 (47.8)	28.9
T.harzianum Th N1	85.0 (67.2)	60.0 (50.7)	23.3
T.harzianum Th N2	100.0 (90.0)	45.0 (42.1)	40.0
P.fluorescens Pf 3	100.0 (90.0)	60.0 (50.7)	23.3
P.fluorescens Pf 4	80.0 (63.4)	75.0 (60.0)	6.7
P.fluorescens Pf 2	80.0 (63.4)	30.0 (33.2)	56.7
Pathogen check	70.0 (56.7)	90.0 (71.5)	
CD (p=0.05) , CV (%) 2.8, 3.5			
Values are mean of 4 replications; Figures in the parentheses are arc-sine transformations			

ICAR-INDIAN INSTITUTE OF VEGETABLE RESEARCH, VARANASI

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A. DIVERSITY

Study on identification and distribution of races among the FOL isolates infecting tomato in India

Thirty nine isolates of *Fusarium* species were maintained in the PDA slants, sterile water, whatman filter paper strip and silica gel. Twenty isolates of FOL were subjected to the PCR amplification using universal primer pair, specific to *Fusarium oxysporum* f.sp. *lycopersici* and *F. o. radicis-lycopersici* based on the polygalacturonase gene. In this study we identified that FOL race 1 is the predominantly infecting race on tomato in India causing wilt disease. For the race identification among FOL isolates, inoculations on tomato differential lines were initiated to validate the molecular based identification. Twenty FOL tomato isolates were inoculated on the cv. Bonny Best, a susceptible line to all the 3 races of the pathogen and found that all the isolates were able to cause the wilt disease on tomato plants with different grades of disease severity, slight yellowing of leaves to severe wilting and drying of plants. All the tested isolates were pathogenic.

B. DISEASE MANAGEMENT

Evaluation of biocontrol agents, botanicals and chemicals against wilt diseases of tomato and chilli under field conditions

Different components such as talc based formulation of *Trichoderma* isolates (Phyto 1-15), two fungicides and botanicals (Datura and Garlic extracts) have been evaluated against *Fusarium* wilt of chilli and tomato under field conditions (Fig. 34). In tomato, treatments like carbendazim + mancozeb (96%), Phyto 6 (88%), carbendazim (86%) and Phyto-4 and Phyto-9 (71%) significantly reduced the wilt severity compared to untreated control plots (33.1%). Increase in yield was observed by 66% (carbendazim + mancozeb) and 61% (Phyto – 14 and Phyto -10) with corresponding yield recorded were 162.7 Q/ha, and 158 Q/ha and 157.7 Q/ha respectively where as the control recorded 98Q/ha.

Fig. 34 Field evaluation of bio control agents, botanicals and chemicals

C. HOST RESISTANCE

To popularize the grafting technique, grafts were raised with root stock of brinjal (EG219) resistant to nematodes, *Fusarium* and bacterial wilt and scion of tomato (IIVR tomato cv. Kashi Amman) resistant to leaf curl virus. The grafted plants were planted in field and the grafts were well established under field conditions (Fig. 35).

Fig. 35 Establishment of grafted tomato plants under field conditions

ICAR-NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS,MAU

Principal Investigator
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A. DIVERSITY

Characterization of *Fusarium* species associated with chilli wilt.

Molecular diversity and phylogenetic analysis of thirty isolates of *Fusarium* causing wilt of chilli, from various regions of Jammu and Kashmir were analyzed using internal transcribed spacer (ITS) gene regions. The phylogenetic tree constructed using bootstrap neighbour-joining analysis of 30 isolates grouped them into two major clusters. The first major cluster had 24 isolates belonging to *F. oxysporum* f. sp. *lycopersici* while the remaining six isolates were belonging to *F. solani,* grouped into the second major cluster (Fig. 36).

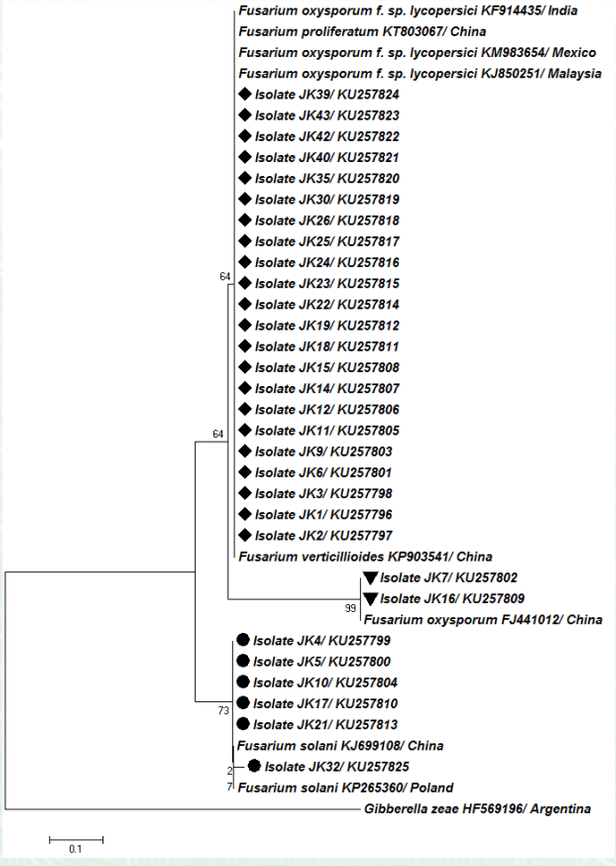

Fig. 36 Maximum likelihood (ML) tree for *Fusarium* isolates based on ITS gene sequences

Genetic diversity analysis by RAPD-PCR analysis

Among the ten RAPD primers, three primers *viz.*, OPA-2, OPA-3 and OPA-11 and OPA-13 were chosen based on their capacity to reveal polymorphisms among isolates. RAPD analysis of genomic DNA from the pathogenic isolates revealed the presence of ten clusters at the arbitrary level of 50% similarity. Maximum isolates were clustered in group II (JK9, JK11, JK14, JK12, JK15, JK24, JK25, JK26, JK30 and JK42) followed by group III (JK18, JK 23, JK35 and JK43).

Development of PCR based diagnostic markers for *Fusarium*

Different gene sequences, included exopolygalacturonase (Pgx), intergenic spacer (IGS) and alcohol dehydrogenase (ADH) gene sequences from related species, genera, and other pathogens were retrieved from NCBI database for the development of genus and species-specific diagnostic marker for *Fusarium* species. PCR assay was performed to assess diagnostic markers for eleven different isolates of *F. oxysporum* f. sp. *lycopersici* along with different species of *Fusarium* and some other unrelated fungi (e.g. *F. solani* FS-10, *F. ciceri* JFOC 31, *F. udum* Akola 1, *Alternaria brassicae* BAB1, *A. brassicicola* OCA10, *Colletotrichum falcatum* Cf 648, *C. gloeosporioides* Cg1, *Trichoderma harzianum, Trichoderma asperellum, Beauveria bassiana* 1, *Beauveria bassiana* 2, *Rhizoctonia solani* 4, *R. solani* 7). The primer pairs designed using Pgx1, Pgx3, Pgx2 and ADH amplified a fragment of ~781bp, ~500 bp, ~620 bp and 1100 bp in different *Fusarium* species, however no band were detected in unrelated species . Similarly, species specific marker using IGS and Pgx5 was developed for FOL. The primer pairs designed from these regions resulted in ~633-, and ~1100 - bp amplicons in all the isolates of FOL and no amplicons were detected in other *Fusarium* and unrelated fungal species.

ICAR-NATIONAL RESEARCH CENTRE FOR BANANA, TIRUCHIRAPALLY

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A. DISEASE MANAGEMENT

Field evaluation of effective microbes and botanicals for Foc suppression and plant growth promotion

A field trial (3rd year) was conducted in 2015 with effective microbes and botanicals in a *Fusarium* hot spot area at Muthulapuram of Theni District, Tamil Nadu, India. In this trial, three effective treatments along with the fungicide carbendazim and control were evaluated for *Fusarium* wilt suppression and plant growth promotion . The tissue culture plants cv. Grand Naine were bio-primed and the treatments were given three times at monthly intervals through soil application (at the time of planting, 2nd month after planting, 4th month after planting). The results indicated that the liquid formulation of endophytic *Bacillus flexus* + endophytic *Trichoderma asperellum* (A) recorded the lowest disease score of 1.7 which was followed by rice chaffy grain formulation of endophytic *Penicillium pinophilum* + rhizospheric *Trichoderma* sp. (B), the Zimmu *(Allium sativum* x *A. cepa)* leaf extract treated banana plants (C) as compared to carbendazim (D) and control (E) which recorded the highest disease score of 5.4 on a disease scale of 1-6 where 1 is healthy and 6 is dead (Fig. 37 a and b). Apart from decreasing the disease severity the above treatments also increased the plant growth parameters such as plant height, girth, total number of leaves and leaf area significantly. The average bunch weight was also significantly higher in liquid formulation of endophytic *Bacillus flexus* (Tvpr1) + endophytic *Trichoderma asperellum* (Prr2) (44 kg) followed by rice chaffy grain formulation of endophytic *Penicillium pinophilum* + rhizospheric *Trichoderma* sp. (38.6 kg), Zimmu leaf extract (36.4 kg) as compared to chemical (23.3 kg) and control (16.2 kg) (Fig. 38 a & b).

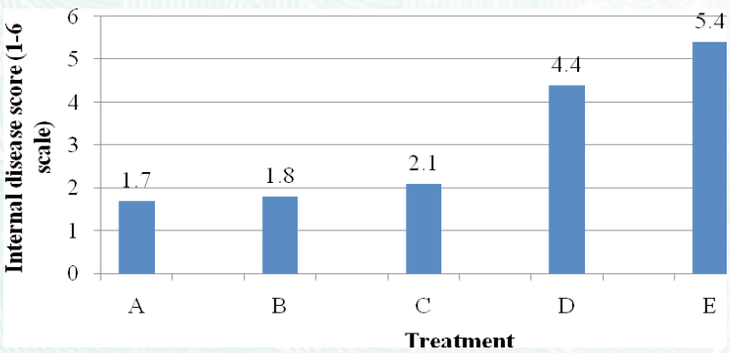

Fig. 37 a. Effect of biocontrol agents on the internal symptoms in corm tissue in banana .

Fig. 37 b. A) Untreated control plants
B) Plants treated with *Bacillus flexus* + endo. *T. asperellum*

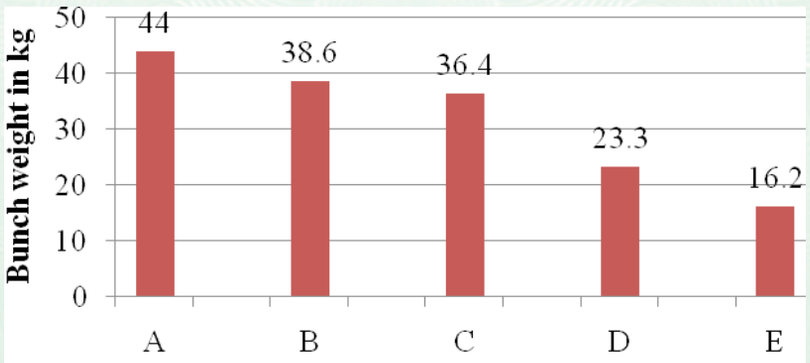

Fig. 38 a. Effect of biocontrol agents on the bunch weight of cv. Grand Naine (AAA)

Fig. 38 b. (A) Untreated (Control)
(B) Treated (endo. *Bacillus flexus* + endo. *T.asperellum*)

Isolation and characterization of volatiles from Zimmu leaf extract

The screening of 33 botanicals against Foc revealed that Zimmu leaf extract @ 50% concentration was found to be very effective in the inhibition of the mycelia and spore germination of Foc. The *in vivo* experiment conducted with Zimmu leaf extract @ 50% concentration also revealed that this extract was effective in controlling *Fusarium* wilt, besides involved in plant growth promotion. Hence, the principle compound particularly volatiles present in the Zimmu was extracted using TA (Tannex)/Hexane. The extracted compound was subjected to GC-MS analysis and the results revealed the presence of a principle compound (PC1) which recorded 100% inhibition of Foc spore and mycelia at 0.1% concentration.

Differential expression of genes in *Fusarium* wilt and biocontrol agent interaction in banana by SSH approach

To identify the differentially expressed genes due to the interaction of Foc pathogen and effective bio control agent in banana, supressive subtractive hybridization (SSH) was carried out in cv. Grand Naine. Out of 300 clones sequenced 258 readable sequences were obtained. among these only 24 sequences were categorized into five groups namely, defense/resistance, signal transduction, transcription, protein synthesis and metabolism. Out of these 24 genes, six defense related genes namely banana lectin- methyl-alpha-mannose complex (mannose binding lectin), calmodulin binding protein, pleotropic drug resistance protein, endochitinase, isoflavone reductase and polyubiquitin were selected for further studies by RT-PCR.

Real Time -PCR analysis of differentially expressed genes

The validation for the six defense related genes performed by RT-PCR in cv. Grand Naine indicated that all these genes were expressed in the root tissues of both Foc alone and Foc + *T. asperellum* inoculated banana plants. However, the expression level of all these genes was consistently higher in Foc + *T. asperellum* inoculated plants as compared to Foc alone inoculated plants. The transcript level of defense related genes endochitinase, mannose binding lectin and polyubiquitin genes has reached the maximum at 5 DPI, whereas the isoflavone reductase and pleotropic drug resistance gene reached maximum level (10 fold) at 7DPI and calmodulin binding protein at 3 DPI in Foc + *T. asperellum* inoculated plants compared to Foc alone inoculated plants. The defense related gene mechanism peaked between 3 DPI and 7DPI in Foc + *T. asperellum* inoculated banana plants.

Comparative proteomics study of pathogenic (Foc VCG 0124) and non-pathogenic *Fusarium oxysporum* (NP-Fo)

Proteomics study of pathogenic *Fusarium oxysporum* f. sp. *cubense* (P-Foc-VCG0124) and non-pathogenic *F. oxysporum* (NP-Fo) strain mycelia proteins was carried out. Functional annotation revealed that the 5 significantly up-regulated proteins are P1-Vesicle transport v-SNARE protein, P2-developmentally regulated GTP-binding protein, P3-ankyrin repeat containing protein, P4-Isocitrate dehydrogenase, P5-Homogentisate 1, 2-dioxygenase and P6-hypothetical protein (unique) respectively .

Comparative proteomics of Tamil Nadu Foc (TN-Foc) and virulent Bihar Foc (Bi-Foc)

Proteomics study of pathogenic *F. oxysporum* f. sp. *cubense* Tamil Nadu isolate (TN-Foc, VCG 0124) and a virulent *F. oxysporum* f. sp. *cubense* Bihar isolate (Bi-Foc) mycelia proteins was carried out. Functional classification of proteins based on Gene ontology revealed that most of the identified proteins correspond to enzymes that are involved in various processes which include carbohydrate metabolism, pathogenicity related process (hydrolase, cellobiohydrolase, exoglucanase A, MAPKKinase, pH response regulator protein, Myb-like DNA-binding domain-containing protein and arylsulfotransferases), protein synthesis, transport and regulation and signal transduction (Fig. 39).

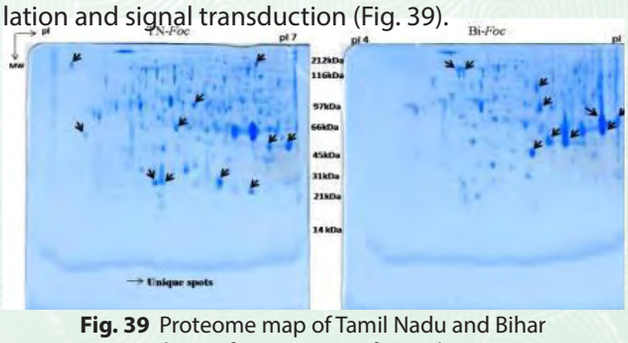

Fig. 39 Proteome map of Tamil Nadu and Bihar isolates of *F. oxysporum f.*sp. *cubense*

ICAR-NATIONAL RESEARCH CENTRE ON SEED SPICES, AJMER

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A. DIVERSITY

The 42 fungal isolates from infected cumin roots collected from different locations Rajasthan, Gujarat and Madhya Pradesh were identified to be *Fusarium* sp. Based on the taxonomic identification made by ARI, Pune out of these 42 fungal isolates 20 were *Fusarium solani,* nine were *F. oxysporum*, three were *F. moniliforme* and eight were unidentified species. All the isolates showed similar trend in colony development but the pattern of pigmentation varied. Dark purple, pink and yellow colour were observed and in few cases dull shades of the three colours were also observed. All the isolates were screened for its interaction with the bio-contol agent *T. asperellum* culture TIF 1 and CU3-01. Dual culture studies showed significant effect of the bio-control agent in control of the fungus. Out of 42 fungal isolates 38 isolates were studied for the ITS sequence variation. The phylogenetic analysis based on ITS sequence showed distinct grouping of the *F. oxysporum* and *F. solani* species . Isolate AFIC-38 (*Fusarium solani*) formed a separate cluster.

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A. DIVERSITY

Disease affected sugarcane samples from the institute field and surveyed areas were subjected to pathogen isolation. About 89 wilt infected samples were subjected to pathogen isolation, in which 73 isolates were recovered. They were sub cultured and added to the existing *Fusarium* culture collections in the lab. Detailed investigations were conducted in National Hybridization Garden (NHG) which houses more than 600 parental materials for the status of wilt, its severity and associated factors in inducing wilt. Clones expressing wilt either alone or in association with root borer, termites, internode and top borer were recorded. About 26 per cent of clones were infected with both wilt and root borer while 56 per cent of clones were infested with other pests and wilt. About 39 per cent of clones recorded root borer infestation alone. The other factors like termites and other pests were recorded in 67% of clones.

B. EPIDEMIOLOGY

Surveys were conducted during the 2015-16 season for the incidence of wilt on a set of genotype /varieties maintained in National Hybridization Garden (NHG) as parents. Based on the expression of disease severity among the 611 clones, wilt was recorded in 132 clones. Early symptoms of wilt like chlorotic discolouration of the leaves and drying were observed during 3-4 months old crop in the cvs Co 94012, Co 7214, Co 88013 and Co 7219. These varieties recorded progressive disease development and eventually complete destruction of the plants during grand growth phase (Fig. 40). The varieties such as MS 68/47, Co 94012, Co 7219, Co 87023, CoT 8201 and Co 7214 exhibited typical wilt and approximately, 75% of the canes of the cvs Co 0240, Co 0120, Co 05011, Co 62174 and Co 87044 showed typical rind paleness and cane drying in the field and discoloration inside the canes.

Epidemiological studies under field conditions

Based on the diseases expression during 2014 -15 disease development in disease affected setts were monitored in 14 varieties. Among the different varieties Co 86002 and MS 901 suffered more severely and

Fig. 40 Progressive wilt development in sugar cane cv Co 72219

recorded more than 75 per cent of wilt incidence from the tillering phase to grand growth phase. The cvs ISH 100, Co 740 and CoT 8201 recorded 60% wilt incidence in the diseased plot. Overall observations indicated that sett-borne inoculum had resulted either reduction of germination or post germination death / drying of settlings during tillering phase as well as grand growth phase in case of the cvs CoOr 03152, MS 901, Co 98010, C79128, Co 86002, CoT 8201 and CoJ 83. As days progressed, most of the diseased plots exhibited gradual yellowing of leaves and wilting of canes than the healthy plot. The suspected leaf and stalk samples

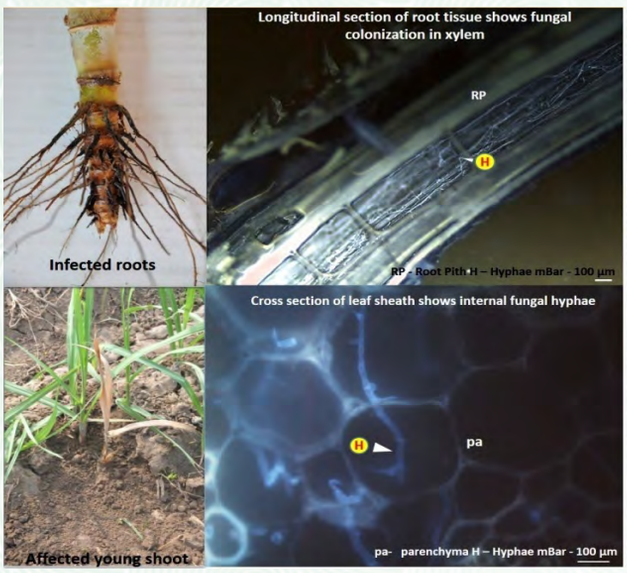

Fig. 41 Histological studies on wilt affected root and leaf

were collected and subjected to histological studies and pathogen isolation (Fig. 41). The pathogen was recovered from 80% by the tissue samples collected from different varieties. The results revealed that, under favourable conditions the wilt inoculum settled in the affected mother setts could act as a source of pathogen inoculum and induced progressive yellowing of leaves, wilting and drying of the plants in all the susceptible varieties.

Simulation of wilt under controlled conditions

The study was initiated to assess the potential of inoculum sources of wilt affected (sick) soil and affected setts on disease initiation in sugarcane and further progress of disease inside disease testing chamber. The rhizosphere soils from the wilted canes were used to create a sick soil condition. Dilution factor of 10^{-2} was chosen as an optimum dilution which resulted in *Fusarium* inoculum load of 3.8 x 10^2 cfu/g. Observations on plant growth at monthly intervals after planting revealed that the cv Co 0238 showed 100 % germination in healthy soil (HS) + healthy cane (HC) pits whereas it was only 25% in case infected soil (IS) + infected cane (IC) pits. Cultural and histological studies of samples from internode, node and roots of infected plants clearly indicated the presence of *Fusarium* systemically. The study revealed that, the varieties respond differentially for wilt development from two different inoculum sources. Further the results of study indicated that the infected soil inoculum played a crucial role in initiation of the disease development as compared to the sett borne inoculum and in susceptible varieties when both sources of inocula were there, disease development was faster .

In the second trial, *Fusarium* infected cane debris was used as a source of inoculum to induce wilt in a set of 10 varieties. The wilt infected canes from the institute field were chopped and incorporated in the soil. The rhizosphere soil from the wilted canes were collected and used as another inoculum source. Ten wilt susceptible clones were planted in these plots. Initial observations indicated that infected cane debris caused severe impact on sett germination in most of the varieties. However sick soil condition showed normal germination but with stunted growth .

C. DISEASE MANAGEMENT

The wilt affected setts of Co 7219, Co 86002, and Co 94012 were treated with fungicide carbendazim 0.1% and planted along with healthy and infected controls in the field. By 60 DAP, a distinct variation in germination and plant height was observed between the fungicide treated and control plots. The results of the trial clearly indicated that fungicide treatment minimized

Fig. 42 Management of *Fusarium* wilt of sugarcane under field conditions

Field trial

The field experiments to manage *Fusarium* wilt using systemic fungicide carbendazim were conducted during 2015-16 season under field conditions. Wilt affected planting materials were treated with fungicide and planted along with the respective healthy and infected controls. Fungicide treatment showed a distinct variation in germination, cane height and cane population than the affected setts (Fig. 42). Although fungicide treatment improved germination in the disease affected setts the germinated shoots exhibited wilt during grand growth phase of the crop. It indicates that sett treatment alone insufficient to manage the sett borne inoculum. Further interventions are required to reduce the diseases development during the grand growth phase of the crop.

Status of pokakh boeng disease incidence in NHG

Detailed observations were conducted during the 2015-16 season for the incidence of pokkah boeng (PB) in NHG where more than 600 clones of sugarcane with different generic background are maintained. During tillering phase to grand growth phase of the crop, symptoms of PB were noticed. As the days progressed the clones affected with PB showed wilting in some cases. Chlorotic symptom began on young leaves or top portion of a plant. Later young leaves in the spindle became twisted, wrinkled and shortened. During the observations, three phases of PB *viz.*, cholorotic phase, acute phase and knife cut phase were noticed (Fig. 43) in the clones CoP 06436, CoV 89101, Co 617, Co 7915, Co 8210, Co 92002, ISH 69, Co 98006, CoA 10321, CoBln 94063, C79180, CoH 70, CoJ 82315, CoLk 97169, CoS 109, NB 94-545, CoJ 64 and CoJ 83 initially showed PB symptoms, later they recovered and remained disease free. Overall, of the 611 clones, 162 clones were affected with different intensities of PB at early stages of the crop. Among the 162 were clones, 51 clones showed wilting of the cane.

Fig. 43 Different phases of pokkah boeng disease

Pathogenicity of *F. sacchari* isolates by detached leaf assay

The fully opened young leaf (3rd) of susceptible cv CoC 671 was used for this study. Before inoculation, the upper surface of the leaves was wounded by pin prick method and the cut leaves were placed in a moist chamber. The injured spots on the leaves were inoculated with *F. sacchari* ($1x10^6$) as spore suspension along with sterile water as control and incubated at 22°C. In the detached leaf bioassay, the fungal inoculum caused a necrotic lesion on the leaves (Fig. 44). Under microscopic observation of *Fusarium* inoculated leaves revealed that the pathogen conidia were able to germinate and formed primary mycelium and hyphal colonization. The assay will be used further to screen the *F. sacchari* isolates for variation in their pathogenicity.

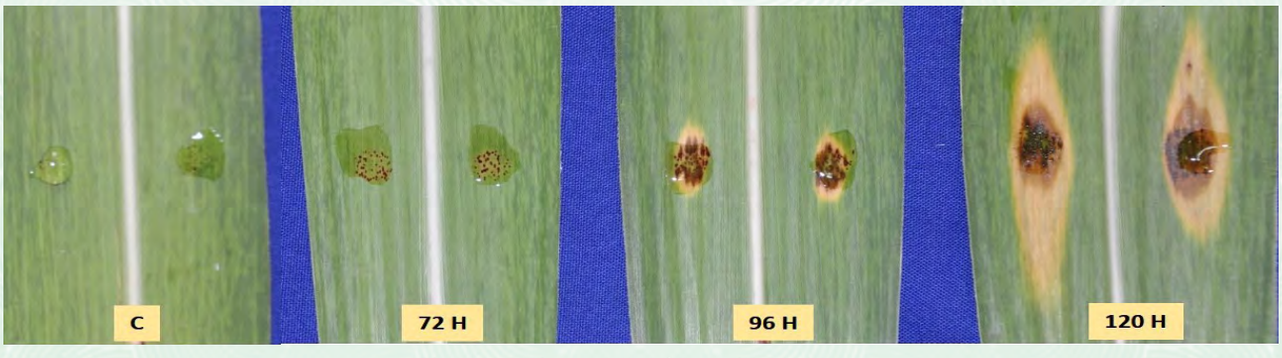

Fig. 44 Pathogenicity of *F. sacchari* isolates

ICAR-INDIAN INSTITUTE OF PULSES RESEARCH, KANPUR

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Co-Investigators :
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Dr. K R Soren

A. DIVERSITY

Diversity in *F. oxysporum* fsp. *ciceri*

Fifty nine isolates of *F. oxysporum* f.sp. *ciceri* were inoculated on to 14 differential genotypes of chickpea (JG 62, CPS 1, C 104, JG 74, JG 315, BG 212, K 850, Chaffa, KWR 108, DCP 92-3, GPF 2, Annegiri, L 550, IPC 2004-52). Isolates could be categorized in to seven races . Majority of the isolates (19 nos.) could be grouped in to race 2, whereas 14 isolates were grouped in to race 5. Only one isolate resembled race 1.

B. DIAGNOSTICS

Molecular characterization of isolates of *F. oxysporum* f.sp. *ciceri* and *F. udum*

16 isolates of Fusarium Oxysporum f.sp. ciceri and 15 isolates of *F. udum* were analysed to identify the phylogenetic relationships among them. Based on 31 sequences, phylogenetic tree was constructed for grouping different species of *F. oxysporum* and *F. udum*. The level of genetic variation observed in the SSU data set ranged from 0.4 to 15.6% and showed intergenic variation of SSU sequences between *F.udum* as well as Foc. The conservation of the mitochondrial SSU rDNA sequences within species and the degree of inter and intra specific variation found in the *Fusarium* species will facilitate to use these sequences as specific molecular marker.

C. HOST RESISTANCE

Chickpea

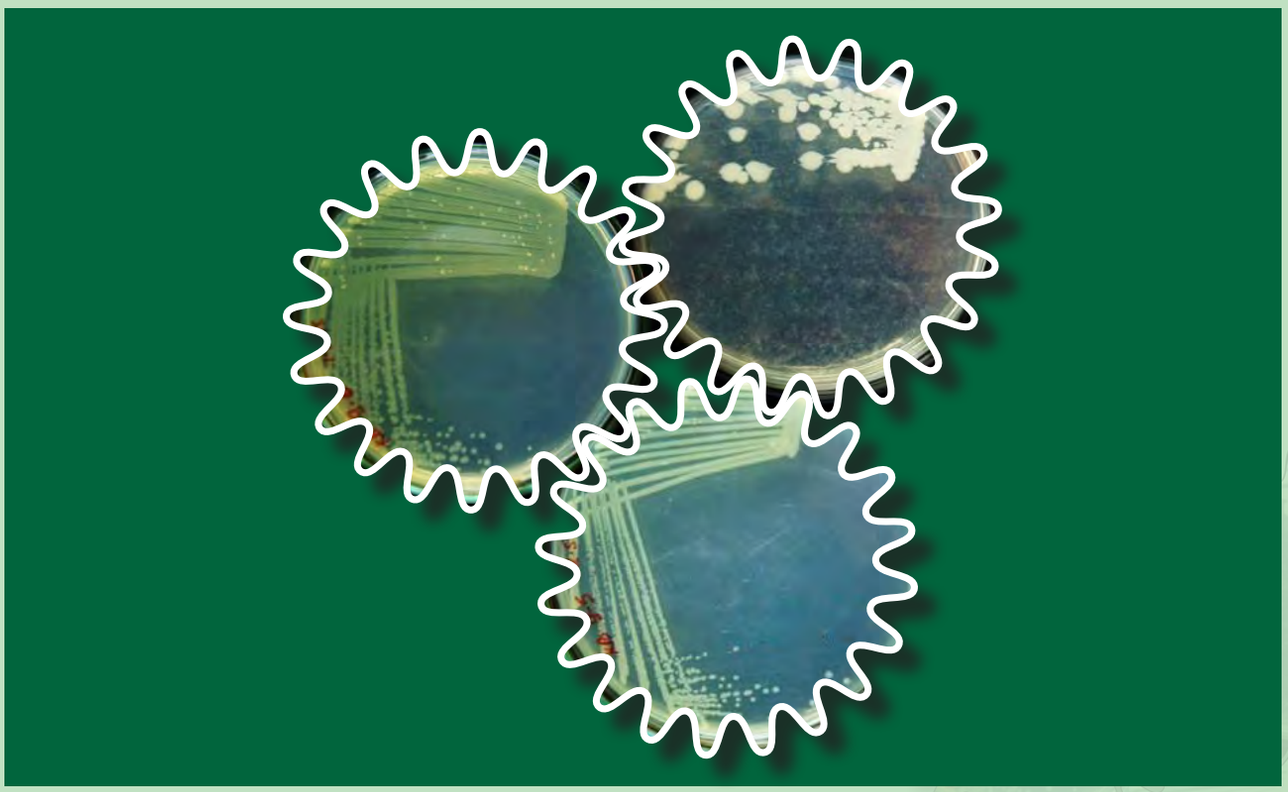
One hundred twenty five chickpea accessions were screened against wilt in a sick field where the susceptible check (JG 62) showed 100% mortality. Twenty one of them were resistant whereas 15 were moderately resistant remaining and 89 were susceptible. Out of 75 IIPR lines screened, 31 were found resistant and 18 lines were moderately resistant. Among 20 kabuli entries only 2 were found resistant (IPCK 2015-235 and IPCK 2015-264).

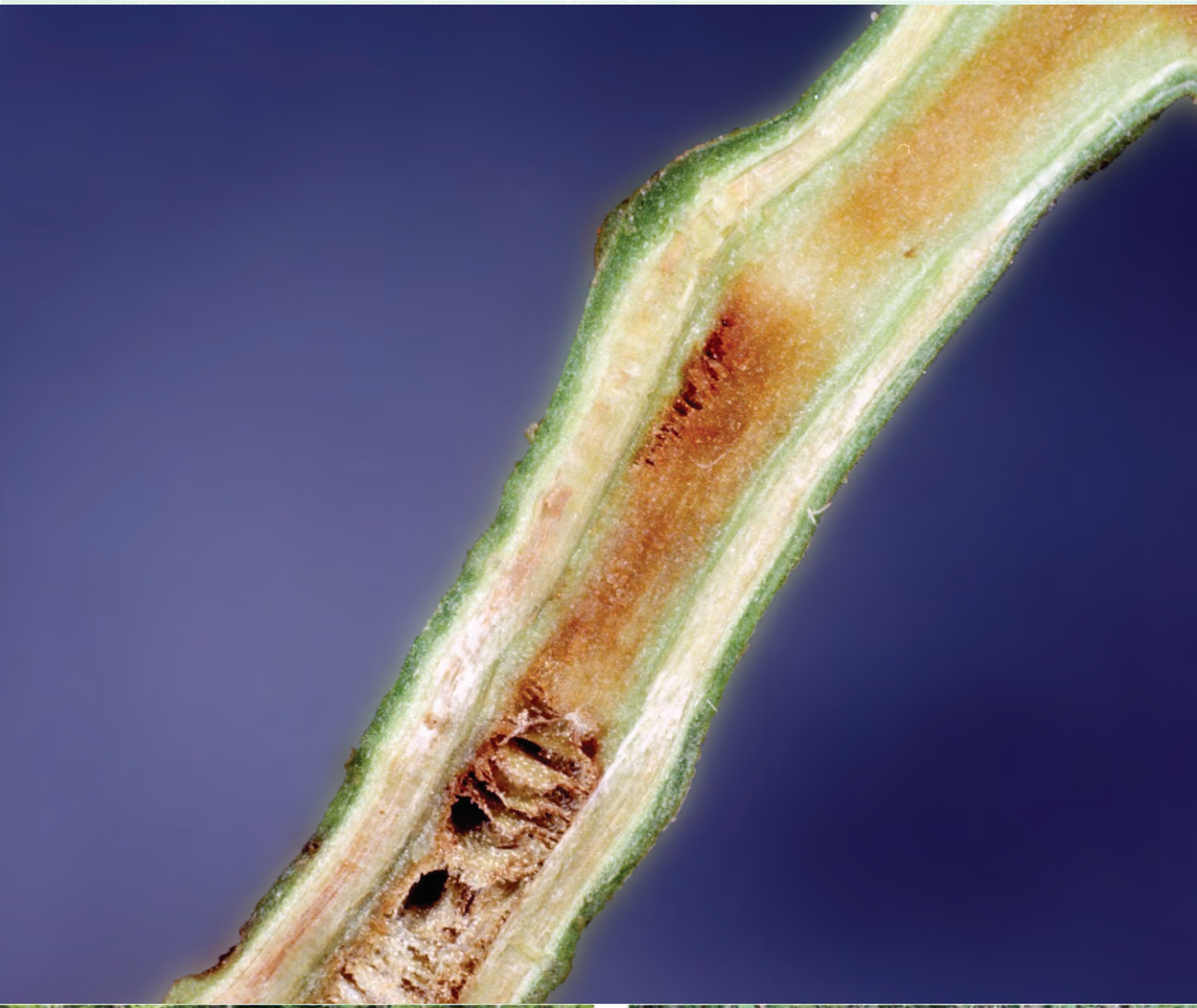
Genotypes found promising under field screening were screened against six races of *F. oxysporum* f.sp. *ciceris* in separate cement pots containing inoculum of a each race. Of the 25 promising genotypes of chickpea, IC-83603, IPC2005-24, IC-83587, IPC2005-34 and IPC2005-15 were found resistant to all the six races. IC-83614, IPC2005-27, IPC2005-26, IPC2005-30, IPC2005-37, IPC2005-19, IPC2005-35, IPC2004-08, IPC2005-43 and IPC2004-03 were resistant to five races. IPC2005-18 was resistant to four races. IC-83563, IC-83601 and IC-83583 were resistant to three races and IC-83560, IC-83559, IC-83567 and IC-83565 were resistant to two races. One genotype IC-83592 turned out to be susceptible to all six races.

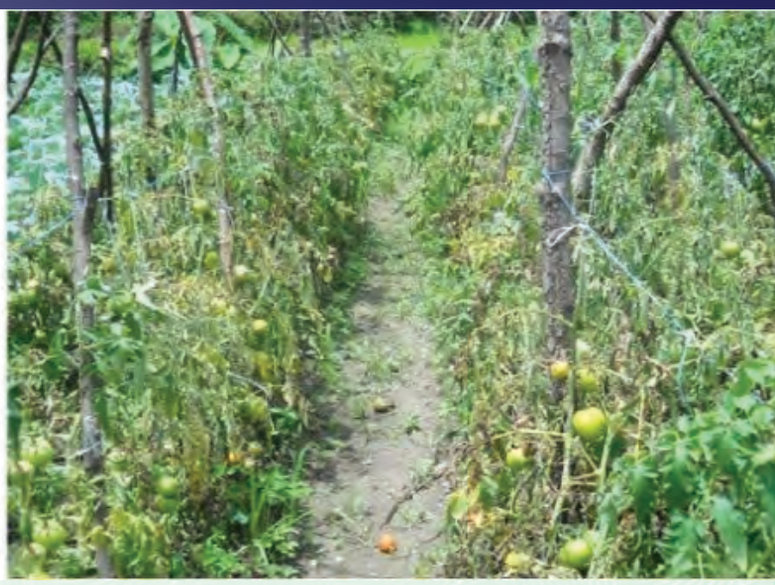
Pigeon pea

Total 402 pigeon pea lines were screened against *Fusarium udum* in wilt sick plot wherein pigeon pea variety Bahar was used as susceptible check showed 100% mortality. IIPR promising lines *viz*, DPPA 85- 3, DPPA 85-5, DPPA 85-7, DPPA 85-8 DPPA 85-11, DPPA 85-12, DPPA 85-13, DPPA 85-14, DPPA 85-16 and IPA-38, IPA 16F, and IPA 15F continued to show resistant reaction. Genotypes, ICPL99044, ICPL 20095, ICPL 87051, ICPL 99009, and ICPL 99055 from ICRISAT were found resistant. Twenty nine genotypes of pigeon pea used as donors in the resistance breeding programme were planted in the wilt sick plot to monitor their reaction. Most of the donor lines continued to show resistant reaction.

Ralstonia







ICAR- CENTRAL COASTAL AGRICULTURAL RESEARCH INSTITUTE, OLD GOA

Principal Investigator :
Dr. D Ramesh

A. DIAGNOSTICS

Validation of LAMP PCR for detection of *R. solanacearum*

LAMP PCR was standardized to detect the bacterium directly from soil and plant tissues. The detection technology was validated using the field soil samples. Soil samples collected from the region surrounding wilted plants and apparently healthy plants of brinjal from different fields were used for validation studies. LAMP-PCR with agarose gel electrophoresis was found to be the best method (100% detection) to detect *R. solanacearum* followed by plating on SMSA medium.

B. GENOMICS

R. solanacearum, mutants of hrcV and hrpB genes was developed by insertion mutagenesis. Based on the whole genome sequences, 3 effector genes of *R. solanacearum* were selected, cloned into destination vector and transformed to wild type *R. solanacearum* to study the translocation of effector in the wild type and mutants.

Transcriptome analysis of brinjal

Resistant and susceptible brinjal varieties were used to develop the complete transcriptome data to identify the genes involved in resistant reaction when the pathogen infects the plant. Plants were challenge inoculated and samples were collected at different intervals were taken.

Seed transmission studies of *R. solanacearum* in brinjal

Bacterial wilt is a major yield constraint of brinjal in coastal region and mainland parts Experiments were conducted to study the seed transmission of the bacterium through external and internal methods. The seed wash and seed macerate were tested by plating and PCR using specific primers. Twenty two seed samples were tested in the experiment after 15 days, five months and eight months of seed extraction. Results indicated that no *R. solanacearum* was detected in plates, and in PCR test. Seeds sown in under greenhouse were found to be free from wilting. This study confirms that *R. solanacearum* is not carried to seeds from the diseased brinjal plant.

C. HOST RESISTANCE

Development of mapping population for bacterial wilt

Fig .45 Variation in colour in F6 generation of brinjal

Fourty six lines of F5 and 222 lines of F6 which vary in fruit colour, fruit shape, fruit size, colour pattern and bearing habit are grown under field conditions for phenotyping bacterial wilt resistance in the next generation (Fig. 45).

D. DISEASE MANAGEMENT

Grafting of cultivated brinjal on wild brinjal

Locally preferred brinjal cultivars, Agassaim and Taleigao are highly susceptible to bacterial wilt and a wild relative of cultivated brinjal is identified as resistant to this disease. Seedlings of the wild type and cultivates species were raised and the susceptible plants were grafted on the wild types. Results showed that brinjal (cv. Agassaim, Taleigao) and segregating population F4 grafted on wild brinjal are completely resistant to bacterial wilt. However, non-grafted seedlings planted in adjacent rows showed severe bacterial wilt (66-83%). Per cent wilt incidence was significantly different at both 5% and 1% level of significance between the grafts and seedlings. Grafted plants produced the similar type (size, colour) of fruits as that of fruits from non-grafted seedling plant. Number of fruits per plant is more compared to non-grafted seedlings. Yield of grafted plant is very high compared to non-grafted seedlings.

Management of bacterial wilt using biocontrol agents

Six strains of biocontrol agents identified to be effective in managing wilt in brinjal under greenhouse conditions were evaluated in field. Four strains of xylem residing bacteria (XB86, XB122, XB102 and XB177) and two strains of endophytic bacteria (EB69 and RP7) were applied as talc formulations in the nursery and also during planting. All the antagonistic bacteria colonized (6.0 Log CFU g-1 of soil) the rhizosphere of brinjal at 30 days of treatment in the nursery. RP7 colonized the stem and XB177 colonized stem and leaf tissues. Incidence of wilt was recorded regularly. Wilt started 30 days after planting and continued till the end of the crop duration. The lowest wilt incidence (3.33%) was recorded in EB69 treatment. XB177 and XB122 recorded less than 10 per cent wilt where as control showed 41 per cent wilt incidence. The highest yield was recorded in EB69 (45.5 t/ha), XB122 (38.21 t/ha), XB177 (33.35t/ha) and RP7 (32.78t/ha) treatments. Biocontrol efficiency (BCE) and increase in yield was higher in EB69 (92% and 104%), XB177 (84% and 50%), XB122 (80% and 71%) and RP7 (64% and 47%) treatments.

ICAR-CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA

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Dr. B P Singh

Co-Investigators:
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Dr. S Sundaresha and Dr. V U Patil

A. HOST PATHOGEN INTERACTION

Development of bacterial wilt resistant transgenic lines through RNAi approach

To isolate candidate genes required for susceptibility (plant disease susceptibility factors) or create knock-down plants showing a disease-resistant phenotype was attempted. Virus induced gene silencing (VIGS) screening of genes related to disease susceptibility using potato variety "Kufri Jyoti" and the tobacco rattle virus vector system (pTRV2). The *Ralstonia solanacearum* responsive genes *ie.,* PAP2 gene were randomly cloned into the pTRV2 vector and transformed into *Agrobacterium tumefaciens,* and then inoculated into K. Jyoti to create VIGS plants. VIGS plant that barely showed wilting symptoms after inoculation was screened with the pathogen *R. solanacearum*. Resistant plant showed accelerated cell death, rapid accumulation of reactive oxygen species (ROS) (Fig. 46 a). To authenticate the silencing and resistant nature of the VIGS plants, performed qRT-PCR that showed reduced expression of PAP2 and increased expression of FGS2 (Flagellin Sensing receptor gene, Fig. 46b) to achieve effective resistance against *R. solanacearum*. RNAi construct was developed for developing resistance against bacterial wilt.

Fig. 46 a Characteristic symptoms of bacterial wilt in VIGS and control plants

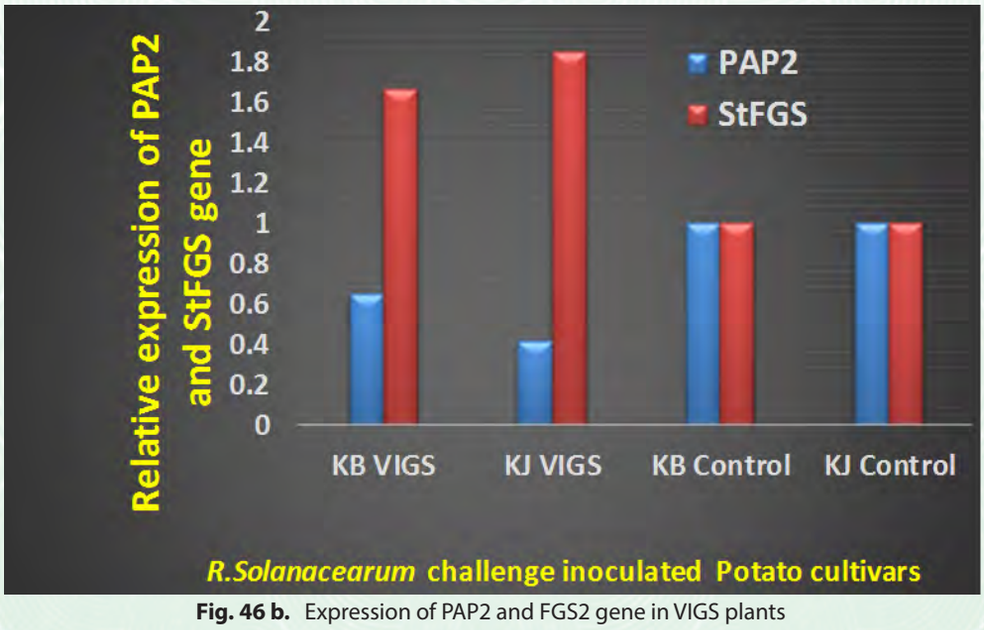

Fig. 46 b. Expression of PAP2 and FGS2 gene in VIGS plants

ICAR-INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Principal Investigator:
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Co-Investigator:
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A. DIVERSITY

Diversity analysis of *R. solanacearum* using molecular methods and biochemical methods

Six isolates of *R. solanacearum* isolated from chilli and capsicum grown in Jammu & Kashmir, Meghalaya, Goa and Karnataka. The biovar of *R. solanacearum* isolates was identified by utilization of disaccharides and hexose alcohols. The result of the biovar test showed that all seven groups of *R. solanacearum* isolates oxidized disaccharides (sucrose, lactose, maltose) and sugar alcohols (mannitol, sorbitol and dulcitol) within 3-5 days. The oxidation reaction was indicated by the change of colour and the all six isolates of groups of *R. solanacearum* belonged to biovar 3. Phylotype affiliation of each isolates was determined all the isolates of *R. solanacearum* belonged to phylotype I *R. solanacearum*.

For molecular characterization, primer pair of 16 s rRNA PCR was used, to confirm *R. solanacearum* Y2 (5- CCCACTGCTGCCTCCCGTAGGAGT -3) and OLI1 5-GGGGGTAGCTTGCTACCTGCC-3). All six isolates belonged to *R. solanacearum* and the primer was found effective to further confirm the isolates.

Multi locus sequence typing (MLST)

In multi locus sequence typing, three virulence-related genes were directly (egl) sequenced. Sets of primers used to amplify internal fragments of these virulence-related genes as egl (840 bp), fliC (390 bp), Hrp_rsB (323 bp). Combined phylogenetic analysis of egl, fliC and hrpB gene of *R. solanacearum* formed 2 clusters, Cluster I representing all reference strains of *R. Solanacearum*, Cluster II represented biovar 3 & 4 i.e KC-14, GC-13, JKC-23, SC-6, SC-7, SC-8 (Fig. 47).

A series was used to identify the selective pressures on virulence-related genes of *R. solanacearum*. First the dN/dS ratios were determined and the values of dN/dS- 1, dN/dS >1, and dN/dSof <1 indicated neutrality, diversifying selection and purifying selection, respec-

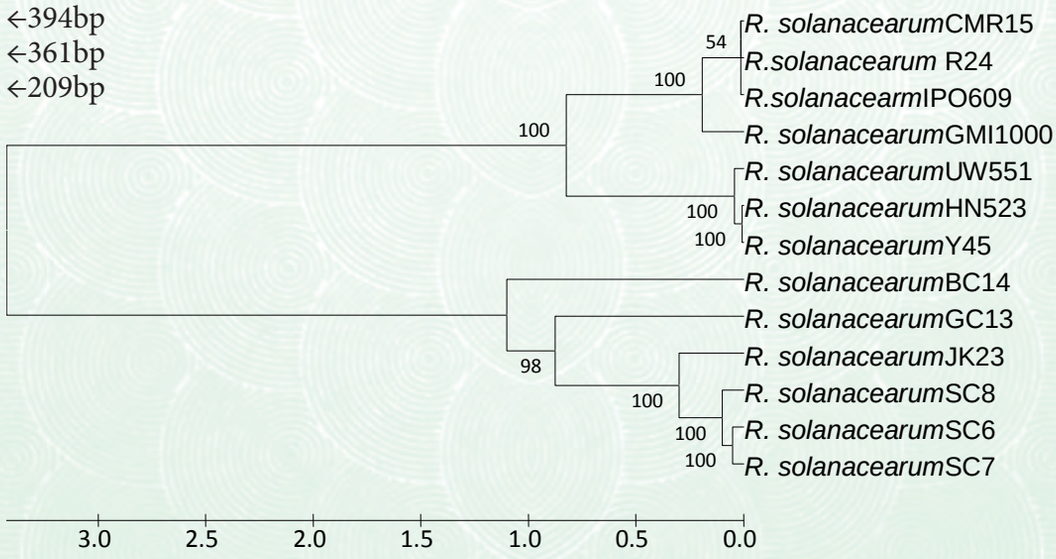

Fig. 47 Combined evolutionary relationships based on HrpB, egl and fliC sequences of 13 isolates of *R. solanacearum* of India

tively, fliC and egl genes showed low levels of dN/dS indicating that these loci are under strong purifying selection conditions. In contrast, dN/dSratio for hrpB was 2.87suggesting diversifying selection is determining the evolution of hrpB genes.

B. DIAGNOSTICS

LAMP assay

The strain UTT-25 of *R. solanacearum* (race 1) was isolated from bacterial wilted tomato plants from Uttarakhand state of India on TTC medium. The LAMP primers were designed based on the six distinct regions (F3, F2, F1, B3, B2 and B1) on the target sequence of egl gene of *R. solanacearum*. A reliable and rapid pathogen detection protocol that utilizes colorimetric LAMP was developed for detection of *R. solanacearum* incited bacterial wilt of solanaceous crops. Four LAMP primers (*i.e.* F3, B3, FIP and BIP) together with PCR primers (F and B) were designed based on conserved sequence of egl62 gene which conserves in *R. solanacearum*. In the present study comparative detection of *R. solanacearum* strain (UTT-25) was done on the basis of conventional-PCR, Lamp-PCR and. Furthermore, the results demonstrated that the LAMP assay was more sensitive and faster as compared to PCR. Specificity of *R. solanacearum* was detected along different other species of bacteria with endoglucanase gene (egl) *viz., Burkholderia glumae, Pseudomonas fluorescense, Erwinia* sp., *Pantoea* sp., *Bacillus subtilis*. It was found that only *R. solanacearum* was amplified and other bacterial species. did not amplify.

The simple colorimetric assay was performed with amplified product which can be visualised by the naked eye either as turbidity or in the form of a colour change when mixed with HNB (hydroxynaphthol blue), a fluorescent dsDNA intercalating dye. A positive LAMP reaction was noticed by a sky blue colour and the colour remained violet for negative reactions . The detection of *R. solanacearum* from the soil, oozing sample, crude sample and asymptomatic tomato plants was also done with egl gene. The LAMP assay optimized in this study was specific and highly sensitive for identification of strains of *R. solanacearum*. The LAMP assay can potentially be used for preliminary screening of *R. solanacearum*.

C. EPIDEMIOLOGY

Interaction between root knot nematode

Table 4. Bacterial wilt incidence in tomato after inoculation with *R. solanacearum* and root knot nematode

Treatments	Disease incidence (%)	Length of plant (cm)	Population	
			R. solanacearum log value cfu/ g of plant tissue	*M. incognita*
N-2000 + 0.1 *R. solanacearum*	85.33a	38.40j	6.04c	46.66b
N-2000 + 0.01 *R. solanacearum*	68.30c	39.36i	5.75e	36.33c
N-2000 + 0.05 *R. solanacearum*	65.70cd	48.02b	5.46hi	46.66b
N-2000 + 0.005 *R. solanacearum*	33.63h	49.43a	5.41ij	23.33ef
N-1500 + 0.1 *R. solanacearum*	78.96b	42.23g	6.05bc	44.66b
N-1500 + 0.01 *R. solanacearum*	65.23cd	41.50h	5.93d	23.33ef
N-1500 + 0.05 *R. solanacearum*	62.20e	46.46d	5.66f	33.23cd
N-1500 + 0.005 *R. solanacearum*	32.26i	49.33a	5.63fg	21.66f
N-1000 + 0.1 *R. solanacearum*	77.96b	43.40f	6.13a	21.66f
N-1000 + 0.01 *R. solanacearum*	64.53de	42.20g	5.77e	20.0f
N-1000 + 0.05 *R. solanacearum*	55.33f	47.53c	5.60g	20.66def
N-1000 + 0.005 *R. solanacearum*	30.96hi	47.66c	5.27k	30.0cde
N-500 + 0.1 *R. solanacearum*	66.66cd	32.56l	6.15a	43.33b
N-500 + 0.01 *R. solanacearum*	39.73g	38.83j	5.75e	49.33b
N-500 + 0.05 *R. solanacearum*	29.56ij	45.56e	5.50h	48.66b
N-500 + 0.005 *R. solanacearum*	27.46j	48.53b	5.37j	43.66b
R. solanacearum (0.1 OD)	67.73c	36.26k	6.11ab	0.00g
M.incognita (1500 J/ml)	0.00k	42.30g	0.00l	60.33a

Means followed by the same letter are not significantly different by LSD test (α = 0.05).

Meloidogyne incognita and *R. solanacearum* on wilt disease in tomato

Thirty days old seedlings of tomato cv. Pusa Ruby were transplanted in six inch autoclaved pots and maintained under controlled conditions. The different juvenile concentrations of *M. incognita* [2000 J2/kg, 1500 J2/kg, 100J2/kg and 500 J2/Kg] with different concentrations of *R. solanacearum* (0.1, 0.5, 0.01 and 0.005 OD) were inoculated at root zone of tomato plants. Observations were recorded 60 days after inoculation, 85.33 per cent of disease was found in the treatment *M. incognita* 2000 j/kg of soil + 0.1 OD of *R. solanacearum* followed by 78.96 per cent in 1500 j/kg + 0.1 OD *R.solanacearum* while in control the 67.73 per cent disease was observed (Table 4).

D. DISEASE MANAGEMENT

Effect of bioagents on hatching of *M. incognita.*

In vitro tests were conducted to determine the effect of bio agents *B. amyloliquefaciens* and *P. fluorescens* on hatching of *M. incognita.* Eggs of *M. incognita* containing second-stage juveniles were maintained at 50 J2/ml. The different serial dilutions [10^{-2}, 10^{-4}, 10^{-8}, and 10^{-16}] of both bioagents were used like at 2, 4, and 8 h exposure and mortality of species were recorded. It was observed that at minimum concentration and at minimum exposure of 2 h more than 95 % mortality of juveniles was noted.

Effect of *M. incognita* and *R. solanacearum* on tomato plants

M. incognita [1500 J2/kg] and *R. solanacearum* [1× 10^8] were inoculated at the root zone of 30 day old seedlings of tomato cultivar Pusa Ruby in different combinations *viz., M. incognita* followed by *R. solanacearum, R. solanacearum* followed by *M. incognita, R. solanacearum* + *M. incognita, R. solanacearum* and *M. incognita* alone. The disease incidence was higher in M. incognita followed by *R. solanacearum,* 86.23 per cent. While disease incidence recorded in treatments, *R. solanacearum* followed by *M. incognita* and *M. incognita* + *R. solanacearum* and *R. solanacearum* alone were 76.26, 68.16 and 66.33 per cent, respectively.

Effect of bio control agents on *M. incognita* and *R. solanacearum* inoculated tomato plants

Thirty days old seedlings of tomato cultivar Pusa Ruby were inoculated with *R. solanacearum* (CFU 1× 10^8) and *M. incognita* 1500 J2/kg followed by the inoculation with the bioagent *B. amyloliquefaciens* (DSBA-11) and *Pseudomonas fluorescens* (DTPF-3) suspension having the (CFU 1× 10^8) near the root zone of tomato plants. The wilt intensity in plants treated with bioagents *B. amyloliquefaciens* + *P. fluorescens* was 26 per cent whereas in plants inoculated with *R. solanacearum* and *R.solanacearum* + *M. incognita* were 66.33 and 72.54 per cent, respectively. The biocontrol efficacy of *P. fluorescens* in combination with *B. amyloliquefaciens* was found higher 64.15 % than the used singly. Plant growth promotion was also observed in treated plants.

Simultaneous detection of polykeptides producing strains *Bacillus* spp. from soil through multiplex -PCR

Total 23 *Bacillus* strains were taken for screening the detection of polyketides genes by multiplex PCR. Primers were designed by using nucleotide sequences of macrolactin, bacillaene and difficidin genes of *B. amyloliquefaciens* and PCR protocol was standardized for the detection of these polyketides producing strains of *Bacillus* spp. with the product size of macrolactin (793 bp), difficidin (705 bp), and bacillaene (616 bp), respectively.

Fig. 48 Multiplex PCR of Soil DNA sample. Lane M. 100 bp DNA Ladder: Lane 1- 2: Jharkhand, 3- 4 :Orissa, 5-6: West Bengal, 7-9 :Jammu & Kashmir, 10:Shillong, 11: Delhi,12-13: Uttarakhand, 14: *B. amyloliquefaciens.*

Detection of polyketides gene in *Bacillus* spp. isolated from soil

The DNA was isolated from soil samples collected from different agro climatic locations of Uttarakhand, Jharkhand, Orissa, West Bengal, Jammu & Kashmir, and Shillong. The soil DNA and standard genomic DNA of *B.amyloliquefaciens* was used to detect the polyketides genes by multiplex PCR. The soil samples from Shillong (Meghalaya) showed higher percentage of polyketides producing *Bacillus* strains (55%) followed by Uttarakhand (46.46 %) and Delhi (40.0%) samples . DNA from Shillong and Delhi were positive for two genes macrolectin and difficidin, while samples from Uttrakhand showed the presence of three polyketides genes. (Fig. 48)

Management of wilt using bioformulation

An experiment was conducted at Chaffi village, (Nainital), Uttarakhand, for the management of bacterial wilt disease in tomato caused by *R. solanacearum* using biocontrol agents *P. fluorescens* and *Bacillus amyloliquefaciens*. Bioagents were applied by drenching and spraying @ 5 g/L during transplanting. After 60 days of transplanting under field conditions the disease incidence was recorded. The minimum wilt disease incidence (15.27%) was recorded in plants drenched with *P. fluorescens* + *B. amyloliquefaciens* whereas in sprayed plants method 24.33 per cent incidence was recorded compared to control (47.55 per cent) (Fig. 49 a and b).

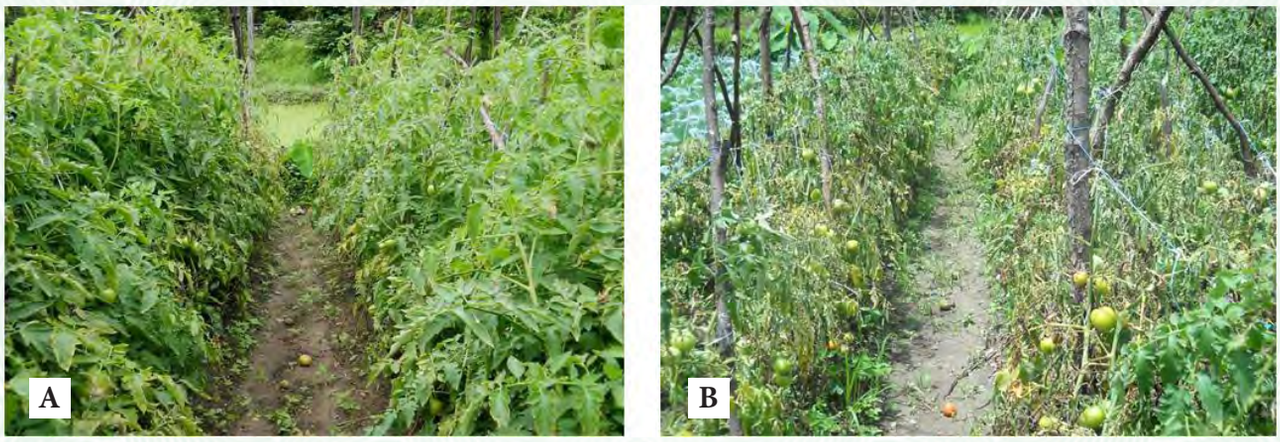

Fig. 49 Effect of biocontrol on incidence of bacterial wilt in tomato
(A) Plants treated with biocontrol agent *P. fluorescens* and *B. amyloliquefaciens* (B) untreated control.

ICAR - INDIAN INSTITUTE OF HORTICULTURAL RESEARCH, BENGALURU

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A. DIAGNOSTICS

Molecular characterization of bioagents (*P. fluorescens* and *B. subtilis*)

Molecular identification with 16S rRNA of potential bioagents (*P. fluorescens* IIRPf24 and *B. subtilis* IHRBs39) which showed maximum inhibition zone against *R. solanacearum* under *in vitro* was carried. The 16S rRNA gene sequences of both the isolates *viz.,* IHRPf24 and IHRBs39 were submitted to the GenBank database of NCBI with accession numbers of KF600748 and KF641179, respectively and the cultures deposited with NBAIM, Mau, with accession nos. *P. fluorescens* IHRPf24-B01474 and *B. subtilis* IHRBs39-B-01475.

B. HOST PLANT RESISTANCE

Studies on induction of resistance by biocontrol agents in tomato

The experiment on induction of resistance in tomato against bacterial wilt through the activity of poly phenol oxidase and peroxidase has been repeated for confirmation. The potential bioagents of *R. solanacearum, viz. P. fluorescens* (IHRPf-24) (Pf) and *B. subtilis* (IHRBs-39) (Bs) were used alone and in combination with pathogen (challenge inoculation) in the induction of defense reactions in tomato. The following six treatments were included in the experiment, *viz.* i) Seeds treated with Bs, ii) Seeds treated with Bs and challenge inoculation with *R. solanacearum* (Rs) 15 days after sowing (DAS), iii) Seeds treated with Pf, vi) Seeds treated with Pf and challenge inoculation with Rs 15 DAS, v) plants inoculated with the pathogen 15 days after sowing and vi) Untreated control check.

For sampling, plants were carefully uprooted without causing any damage to root tissues at different time intervals (0, 1, 2, 3, 4, 5, 7 and 10 days after the pathogen inoculation) to study the induction of defense enzymes in response to pathogen attack in tomato seedlings. Results showed that, seed treatment with *P. fluorescens* induced the plants to synthesize PPO and PO. Increased activities of PPO and PO were observed in *P. fluorescens* pretreated tomato plants challenge inoculated with the pathogen, which remained at higher levels throughout the experimental period. The activity of PPO and PO reached maximum levels of 3.40 and 2.80 OD/min/mg of protein, respectively, on the third day of challenge inoculation with the pathogen.

C. DISEASE MANAGEMENT

Efficacy of bio formulation against bacterial wilt in tomato under glasshouse

Formulation of potential strains of *P. fluorescens* and *B. subtilis* were prepared in the laboratory using talc, farmyard manure and coco peat. *P. fluorescens* and *B. subtilis* cultures were grown separately in nutrient broth for 48 h as shake culture in shaking incubator at temperature 28 ±2°C at 150 rpm. Ten grams of carboxy methyl cellulose was added to 1 kg of sterile talc, FYM and coco peat as carrier material separately and mixed well. About 400 ml of bacterial suspension containing 2.5 x 10^8 cfu/ml was added to the carrier material and mixed well under sterile condition. Fifteen grams of calcium carbonate was added to the formulations to adjust the pH to 7.0. The bio-formulations are being stored under 4°C.

Experiments are being carried out with bio-formulations of *P fluorescens* and *B. subtilis* for the management of bacterial wilt in tomato under glass house condition. The treatments included (i) seed priming

with bio-formulation, ii) seedling dip with bio-formulation, iii) seed priming and seedling dip with bio-formulations, iv) seed treatment and drenching with Streptocycline (200 ppm), v) seed priming with bio-agents (bacterial inoculum) and vi) untreated control.

Development of integrated disease management strategy for bacterial wilt in tomato

Field trials were carried out for the management of bacterial wilt in tomato variety Shivam (bacterial wilt susceptible) with the treatments T1- farm yard manure (FYM) @ 20 t/ha ,T2 -FYM + green manure –sunhemp (GM), T3- Green Manure @ 25 kg/ha, T4- *P. fluorescens* (Pf) – seed treatment and soil application @ 1.0 x 10^8 cfu/ml, T5- *B. subtilis* (Bs) – seed treatment & soil application @ 1.0 x 10^8 cfu/ml, T6- Neem cake @ 150 kg/ha, T7- Pongamia cake @ 150 kg/ha ,T8- FYM + GM + Pf (seed treatment & soil application) + COC 0.2%, T9- FYM + GM + Bs (seed treatment & soil application) + COC 0.2%,T10- FYM + GM + Pf + BS (soil application), T11- Streptocycline 250 ppm + COC 0.2%, T12- COC 0.2% ,T13- untreated control (Table 3). Periodical observations on bacterial wilt incidence were recorded. Results indicated that all the treatments were effective in reducing the bacterial wilt incidence in tomato and increasing the yield as compared to untreated control check. Among the treatments, the bacterial wilt incidence was significantly lowest as 9.54 per cent in plots where combined treatment of FYM + GM + Pf + COC (T8) was imposed. This was followed by the treatments, FYM + GM + Bs + COC (T9) and FYM + GM + Pf + Bs (T10), which recorded 10.20 and 11.26 per cent wilt incidence and were, however, on par with treatment T8. Similarly, the yield was also significantly high in treatment T8 (44.60 t/ha), followed by treatments T9 and T10, which recorded 40.80 and 37.60 t/ha, respectively. However, the untreated control plot recorded highest bacterial wilt incidence of 60.80 per cent and lowest yield of 10.8 t/ha (Table 5 and 6).

Table 5. Effect of various treatments on bacterial wilt incidence in tomato

Treatment	Mean percent wilt*	Per cent decrease in wilt over control
T1	21.50de	64.64
T2	18.70c	69.24
T3	19.00cd	68.75
T4	14.20b	76.64
T5	14.40b	76.32
T6	21.90e	63.93
T7	24.00f	60.52
T8	9.54a	84.30
T9	10.20a	83.22
T10	11.26a	81.02
T11	19.20cd	68.42
T12	20.00cde	67.11
T13	60.80g	--
CD (P=0.05) -2.61,CV(%) -6.92		

* Mean of four replications

Table 6. Effect of various treatments on yield of tomato

Treatment	Yield (t/ha)*	Per cent increase over control
T1	16.82f	35.79
T2	21.15e	48.94
T3	20.20e	46.53
T4	28.00d	64.29
T5	29.20d	63.01
T6	21.40e	49.53
T7	17.00f	36.47
T8	44.60a	75.78
T9	40.80b	73.53
T10	37.60c	71.28
T11	28.40d	51.79
T12	20.80e	48.07
T13	10.80g	--
CD (P=0.05)- 3.19 CV (%) -8.74		

* Mean of four replications

ICAR RESEARCH COMPLEX FOR NEH REGION, UMIAM

Principal Investigator:
Dr. Amrita Banerjee

Co-Investigator:
Dr. G T Behere

A. DIVERSITY

Collection and characterization of *R. solanacearum* isolates

A total of 26 *R. solanacearum* strains were collected from diseased tomato plants as well as rhizospheric soil of solanaceous crops growing in Umiam region of Ri-Bhoi district of Meghalaya during 2015-16. Under *in vitro* conditions all strains produced cream coloured, irregularly shaped, highly fluidal colonies with pink pigmentation in the centre. All isolates were confirmed as *R. solanacearum* with PCR assay and based on utilization of disaccharides and oxidation of hexose alcohols studies, strains belonged to biovar 3. Out of 26 strains, 11 strains isolated from tomato or rhizospheric soil of tomato was tested for pathogenicity on cherry tomato grown in seed trays. The pathogenicity study identified *R. solanacearum* strain (RsRC-S9R1) as the most virulent strain. The virulent *R. solanacearum* strain (RsRC-S9R1) was maintained for further assays.

B. DISEASE MANAGEMENT

Screening of tomato varieties against bacterial wilt

Nine tomato varieties were screened under field conditions against bacterial wilt disease. Wilt symptoms and the number of wilted plants for each genotype was recorded and graded on a 0-5 scale. The maximum wilt incidence (57%) was found in Tomato Selection 3 and minimum incidence (7%) was recorded in Badshah.

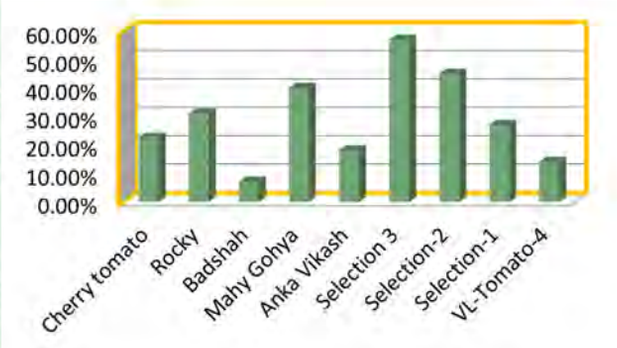


Fig. 50 Incidence of bacterial wilt in different tomato varieties

Isolation of antagonistic organism

Rhizospheric soil from different crops (tomato, potato, sweet potato, brinjal, cauliflower) was collected to isolate antagonistic agents. A total of 29 isolates of *Pseudomonas* spp. were isolated from the rhizospheric soils of different crop plants from two district of Meghalaya; Ribhoi district and East Khasi Hills. The isolates showed variation in colony morphology (Fig. 51). PCR based detection using 16S rDNA confirmed 14 isolates as fluorescent pseudomon as (RCPs-2, RCPs-3, RCPs-4, RCPs-6, RCPs-8, RCPs-9, RCPs-10, RCPs-13, RCPs-14, RCPs-23, RCPs-26, RCPs-30, RCPs-32 and RCPs-34) .

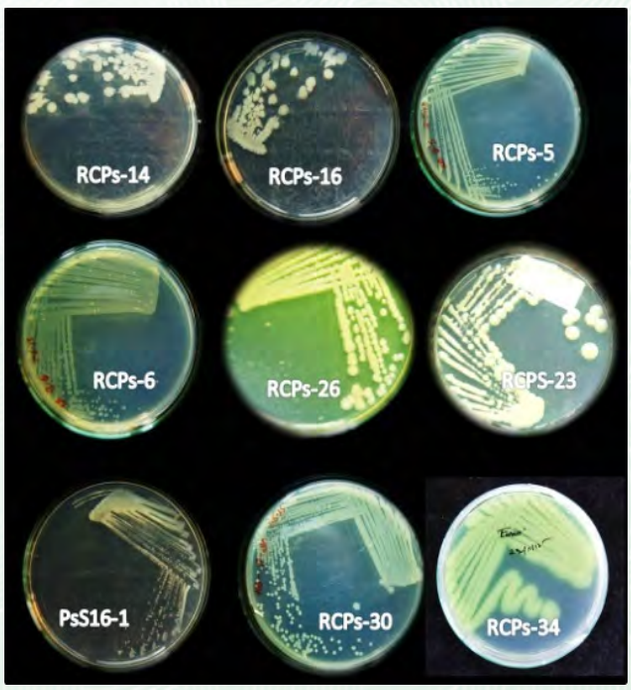
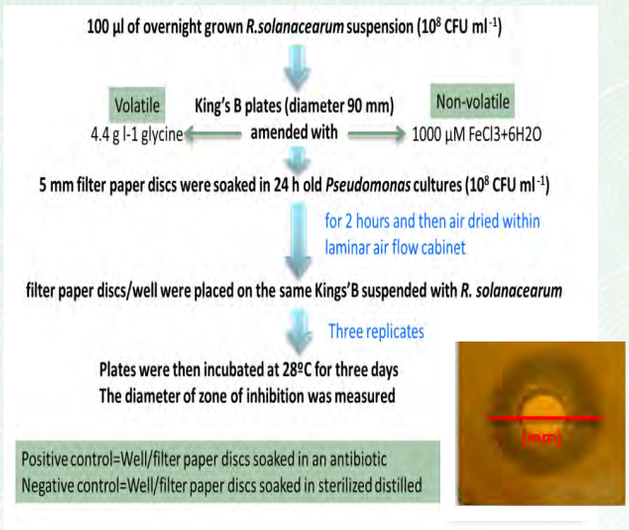


Fig. 51 Colony morphology of antagonists isolated from rhizospheric soil



In vitro antagonist assay

The inhibitory effect of volatile and non-volatile metabolites of 14 fluorescent *Pseudomonas* isolates were evaluated against virulent strain of *R. solanacearum* (RsRC-S9R1) .

Out of the 14 fluorescent *Pseudomonas*, 13 isolates showed inhibition by volatile metabolites whereas, only 6 isolates showed inhibition by non-volatile metabolites (Fig. 52). In case of volatile metabolites, the maximum inhibition zone (19.33 mm) was shown by RCPs-4, while in case of non-volatile metabolites, the maximum inhibition zone (11.0 mm) was shown by RCPs-34. However, isolate RCPs-26 and RCPs-34 showed similar range of antagonism by both volatile and non-volatile compounds.

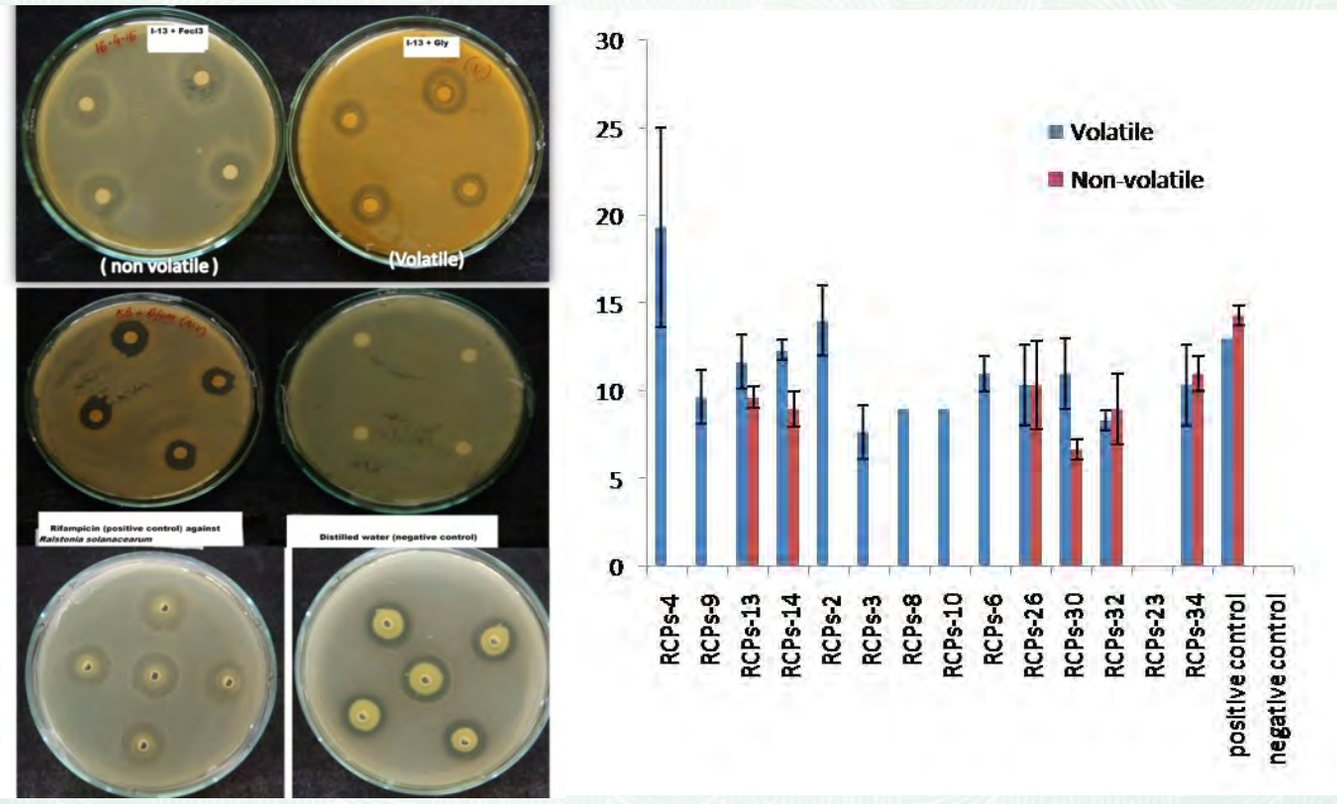


Fig. 52 Effect of volatile and non volatile metabolites of fluorescent *Pseudomonas* against *R. solanacearum* (RsRC-S9R1) under *in vitro* conditions

ICAR –INDIAN INSTITUTE OF SPICES RESEARCH, KOZHIKODE

Principal Investigator:
Dr. D Prasath

Co-investigator:
Dr. R Suseela Bhai

A. DIVERSITY

Five new isolates of *Ralstonia solanacearum* were collected from different bacterial wilt infected fields and tested for cross infectivity and found that none of the isolates from other crops is infective to ginger, while ginger isolates are infective to other crops.

B. DIAGNOSTICS

Loop Mediated Isothermal Amplification for detection of *Ralstonia solanaceraum* biovar 3

A strain specific and sensitive detection methodology using Real Time Loop Mediated Isothermal Amplification (Real Time LAMP) was developed for identification of race 4 strain infecting ginger. Among six housekeeping genes and three virulence genes tested for race specific amplification, gyrB was found suitable for detecting exclusively *R. solanacearum* strains infecting ginger. In Real Time LAMP with genomic DNA of race 4 strains, a sigmoid amplification curve with a Ta value of 92±1°C was observed. The detection limit was found to be 10^3 CFU/g of soil or rhizomes. The method can be used to index soil, water as well as seed rhizomes. Instead of genomic DNA, the extraction of which is cumbersome at field level, the soil supernatant was used as the template in this protocol (Table 7).

Table 7: Sensitivity of real time LAMP by using genomic DNA and bacterial cells

Dilution of genomic DNA (ng)	Amplification time (mm:ss)	Annealing temp (°C)	Titer (CFU/ml)	Amplification time (mm:ss)	Annealing temp (°C)
50	22: 31	92.02	Negative Control (Water)	-	-
5	25:15	92.07	10^9	21:50	92.13
0.5	29:15	92.07	10^8	22:00	92.08
0.05	32:30	92.07	10^7	24:00	92.13
0.005	34:31	92.03	10^6	30:15	92.14
0.005	-	-	10^5	31:30	92.09
0.0005	-	-	10^4	33:15	92.13
0.00005	-	-	10^3	-	-
			10^2	-	-
			10^1	-	-
			10^0	-	-
			Positive	19:00	92.13
			Control (race 4)		

C. DISEASE MANAGEMENT

In planta evaluation of shortlisted apoplastic bacteria against *Ralstonia solanacearum*

A replicated pot trial was carried out with the four shortlisted apoplastic bacteria (IISR GAB 5,IISR GAB 43, IISR GAB 48 and IISR GAB 107) to check their potential against bacterial wilt as well as colonization in the rhizosphere and apoplastic fluid of pseudostems and leaves of ginger plants. To assure the endophytic colonization of apoplastic bacteria, the ginger seed rhizomes were primed in apoplastic bacterial suspension and planted in pots containing potting mixture of soil,

sand and farm yard manure in a ratio 1:1:1. The trial was carried out as CRD with 19 treatments and 3 replications with 3 plants/replication. The treatments include four apoplastic bacteria individually and their combination, copper oxychloride (0.25%), streptomycin sulphate (200 ppm), positive control with pathogen alone and absolute control. *R. solanacearum* GRs Mnt2 (10^8 CFU/ml) was poured in the soil around the base of 45 days old ginger plants. The tiller count in each pot was recorded on the day of pathogen inoculation and the plants were observed for the wilting symptoms. A 100% disease incidence was observed in almost all treatment within 7-20 days except in IISR GAB 107. Within 20 days 90-100 % of plants wilted in pathogen control. A significant reduction in bacterial wilt was recorded only in the case IISR GAB 107. While harvesting, a comparable yield was obtained only in the case of IISR GAB 107 treated plants. Also there is considerable reduction in the population of *R. solanacearum* in the soil treated with IISRGAB 107 when compared with the pathogen control (Table 8).

Table 8 : Evaluation of apoplastic bacteria against bacterial wilt of ginger

Treatment	Disease incidence (40 dai)
Pathogen control	99.98
COC (0.25%)	85.13
Streptomycin sulphate (200 ppm)	99.98
IISR GAB 5	81.66
IISR GAB 43	99.97
IISR GAB 48	72.78
IISR GAB 107	20.98
IISR GAB 5+43	84.91
IISR GAB 5+48	79.36
IISR GAB 5+107	83.58
IISR GAB 43+48	83.24
IISR GAB 43+107	99.97
IISR GAB 48+107	99.98
IISR GAB 5+43+48	99.97
IISR GAB 5+48+107	99.98
IISR GAB 43+48+107	99.97
IISR GAB 5+43+107	99.97
IISR GAB 5+43+48+107	90.94
CV(%)	21.68
SE(d)	15.562
LSD at 1%	42.321

GENOMICS

Whole genome sequencing of *Ralstonia solanacearum*

Two isolates of *R. solanacearum* Biovar 3 race 4 infecting ginger *viz.,* GRs Sikkim and GRsMep2 were whole genome sequenced and the raw data has been assembled using A5-miseq. Both the strains have been annotated using Prokka. In GRs-MEP there are 5120 CDS, 80 tRNA, and 1tm RNA while GRs-SIK possesses 5080 CDS, 63 tRNA and 1 tmRNA. To better classify the predicted proteins from Prokka, a refined annotation has been done using Blast2GO with 1.0E-3 as e-value cut off and 33 as HSP cut off length. The genomes were mined for various effectors proteins and other virulence factors.

Comparative genomics of *Ralstonia* isolates from India

A comparison of whole genomes of 10 isolates of *R. solanacearum* from India *viz.,* GRs-MEP and GRs-SIK of phylotype I from ginger, UTT-25 of phylotype I infecting tomato, RS2, RS25, RS48 and RS75 of phylotype I, IIB and IV infecting potato, Rs-IIHR, RS9 and RS10 of phylotype I infecting brinjal, was taken up. The de-novo assembly of ten *Ralstonia* strains yielded 5.6 to 6.2 Mb genome size at N50 contig length of 58,400 to 68073 bp. Comparative genomics revealed 1463 gene families were conserved among ten *Ralstonia* strains, also the study identified a set of unique genes present in each strain, which confirms that strains are much more diverse among themselves. More than 90% of genes are shared, and gene order is conserved along most of their respective scaffolds in Mummer analysis. Similarly, comparison and MUMi based phylogenetic analysis of these strains with already reported whole genomes of different strains of *R. solanacearum* has been done to determine the relatedness of all available genome pairs. Different numbers and combinations of Type III, IV and VI effectors, cell wall degrading enzymes and genes to overcome the oxidative burst were characterized in these genomes. Significantly, the study provides a deep understanding about the genetic diversity of *Ralstonia* isolates present in the Indian subcontinent.

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2015-16

Name of Institute	Opening Balance (Rs. in lacs)	Amount Sanctioned for 2015-16 (Rs. in lacs)	Total Amount (Rs. in lacs)	Expenditure of 2015-16 (Rs. in lacs)	Closing Balance (Rs. in lacs)
ICAR-CCARI, Goa	7.19	5.00	12.19	6.78	5.41
ICAR-CCRI, Nagpur	11.85	6.00	17.85	14.11	3.75
ICAR-CPCRI, Kasargod	14.18	2.50	16.68	10.41	6.27
ICAR-CPRI, Shimla	1.03	22.00	23.03	20.94	2.09
ICAR-CTCRI, Thiruvananthapuram	0.09	7.00	7.09	6.39	0.70
ICAR-IARI, New Delhi *Fusarium*	1.60	5.00	6.60	5.88	0.71
ICAR-IARI, New Delhi *Ralstonia*	1.62	4.50	6.12	5.79	0.33
ICAR-IIHR, Bengaluru	13.59	9.50	23.09	16.62	6.47
ICAR-IISR, Kozhikode	1.07	10.00	11.07	39.43	-28.35
ICAR-IIOR, Hyderabad	1.13	6.00	7.13	6.90	0.23
ICAR-IIPR, Kanpur	3.57	5.00	8.57	4.82	3.75
ICAR-IIVR, Varanasi	6.52	4.50	11.02	11.92	-0.90
ICAR-NBAIM, Mau	1.63	3.00	4.64	3.77	0.87
ICAR-NRCB, Tiruchirapally	2.86	5.00	7.86	6.82	1.04
ICAR-NRCSS, Ajmer	0.59	2.30	2.89	2.20	0.69
ICAR-RCNEH, Umiam	2.00	6.00	8.00	6.60	1.40
RRII, Kottayam	3.16	3.25	6.42	6.42	0
ICAR-SBI, Coimbatore	1.91	3.50	5.41	4.71	0.70
ICAR-YSPUHF, Kullu	3.92	5.50	9.42	3.96	5.46
Total	79.54	115.55	195.09	184.47	10.62

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भारतीय कृषि अनुसंधान परिषद
भाकृअनुप
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PhytoFuRa
AN ICAR NETWORK PROJECT ON
Phytophthora, Fusarium & Ralstonia

PhytoFuRa Nodal Centre

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