Annual Report
2012/13

भाकृअनुप
ICAR

भारतीय मसाला फसल अनुसंधान संस्थान
Indian Institute of Spices Research
(Indian Council of Agricultural Research)
Kozhikode - 673012, Kerala, India.

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2012/13

IISR Annual Report
2012/13

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद

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भारतीय मसाला फसल
अनुसंधान संस्थान
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Indian Institute of
Spices Research
Kozhikode

Indian Institute of Spices Research

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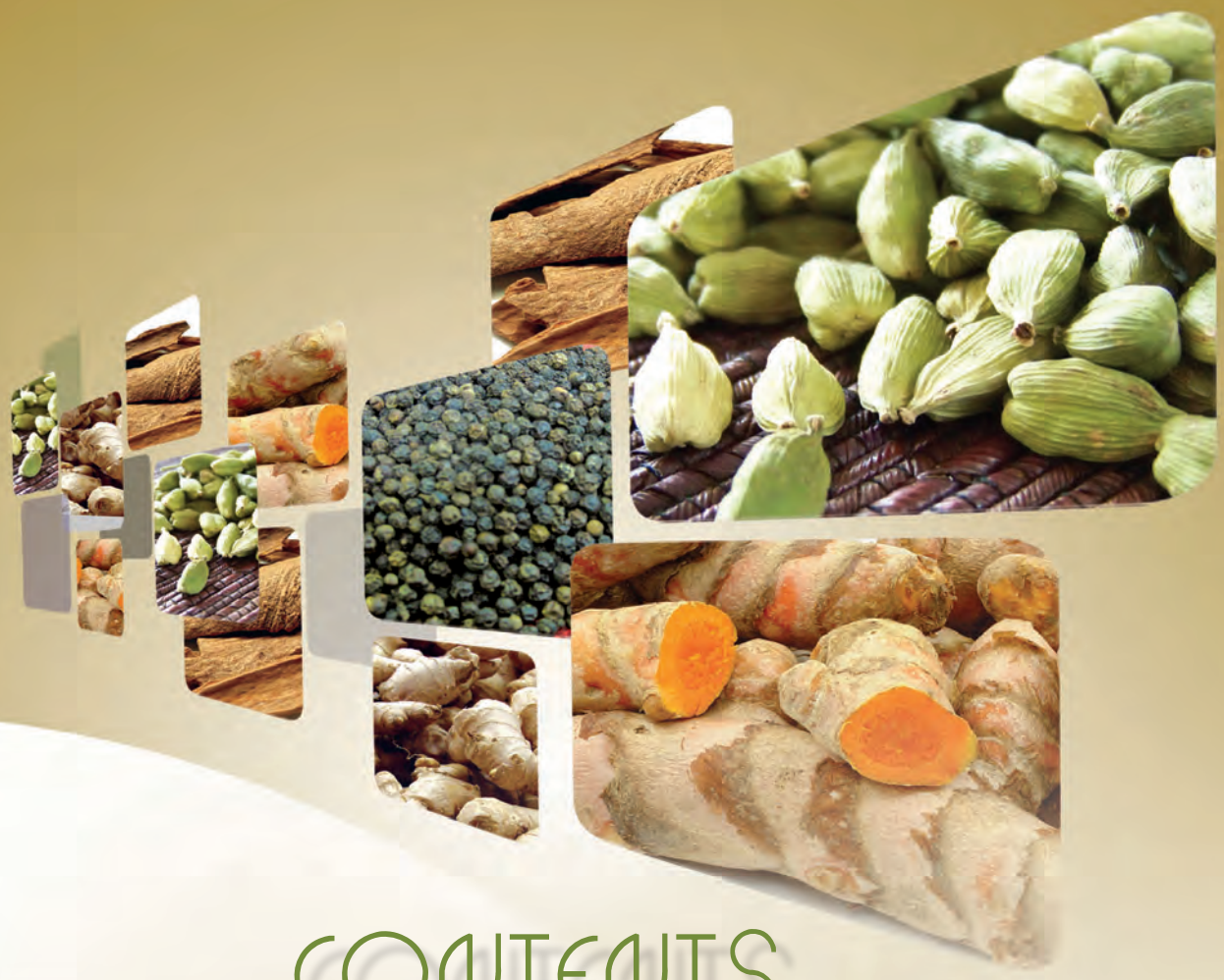

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PREFACE

I have great pleasure in presenting the Annual Report 2012/13. During this year, the institute enriched the germplasm collections in black pepper from Kannur, Malappuram and Idukki districts of Kerala and Kodagu district of Karnataka. Farmer selected cultivars, drought tolerant and long spike types were among the unique collections. Evaluation of new generation chemicals and actinomycetes against *Phytophthora* and nematodes of black pepper and cardamom thrips have given good leads. Liquid formulation for biocontrol agents have been standardized. Technology for control of anthracnose disease in black pepper nursery was developed and recommended.

A quick detection assay based on loop mediated isothermal amplification (LAMP) was developed for detection of Pepper yellow mottle virus (PYMoV) and Cucumber mosaic virus (CMV) infecting black pepper and Banana bract mosaic virus (BBrMV) infecting cardamom. A SYBR Green based method for real-time quantitative RT-PCR (qRT-PCR) for detection of Cardamom mosaic virus (CdMV) and BBrMV infecting cardamom was also developed. The endosymbiont, *Wolbachia* on cardamom thrips was documented. Source-sink relationship and biochemical characterization of resistance for shoot borer have been worked out in ginger and turmeric. The functionally annotated subtracted transcriptome revealed defense/stress related genes like glutathione-s-transferase, leucine rich protein and various enzymes involved in anti-oxidant defense of *Curcuma amada* against *Ralstonia* infection.

A Kisan Mela and Farmers-Scientists interaction meet was organized at Cardamom Research Center (CRC), Appangala during the third week of December 2012. About 1700 soil samples from Wayanad and 10110 soil samples from Kozhikode have been analyzed for all the essential nutrients and soil health cards with nutrient advisories were given. Front Line Demonstrations (FLDs) on varieties and technologies of black pepper were conducted at Wayanad. The Krishi Vigyan Kendra (KVK) conducted 9 seminars, participated in 8 Kisan Melas and exhibitions and trained more than 5150 beneficiaries. Participatory seed production on high yielding varieties of ginger and turmeric was taken up in 10 farmer's plots and public-private participatory seed production of IISR Prathibha turmeric has yielded dividends to farmers in Andhra Pradesh and Kerala. The institute has participated in 3 national and 4 state level exhibitions, organized training programmes to about 100 horticultural officers and farmers of Uttar Pradesh, Andhra Pradesh, Arunachal Pradesh, Karnataka and Kerala. Media visits to progressive farmer's plots were organized and technologies were popularized through video films, AIR programmes and print media.

I consider it a privilege to place on record the encouragement given by Dr. S. Ayyappan, Director General, ICAR during his visit to CRC, Appangala. But for the strong support and guidance we received from Dr. N.K. Krishnakumar, Deputy Director General (Horticulture) and Dr. Umesh Srivastava, former ADG (Hort.II) and Dr S.K. Malhothra, the present ADG (Hort.II) we would not have made these achievements. I am equally thankful to the Research Advisory Committee and the Quinquennial Review Team for their suggestions. I appreciate the efforts shown by the staff of this Institute for their zeal in executing the programmes. I also appreciate the editors for having compiled and brought out this Annual Report.

M. Anandaraj

Director

Kozhikode

Date: 15.06.2013

EXECUTIVE SUMMARY





BLACK PEPPER

Genetic resources

Diverse cultivars of black pepper were collected from farmers' plots in Kannur, Kozhikode, Malappuram and Idukki districts of Kerala. A total of 184 collections were made including 7 wild *Piper* species. Spike proliferating cultivar *Thekkan*, other farmer selected cultivars such as *Kumbakkal* and *Ponmani* were some of the unique accessions collected from Idukki district. A drought tolerant type was collected from Malappuram district. IC numbers were obtained for 191 cultivars and 169 wild accessions from NBPGR, New Delhi. Germplasm accessions were planted at the alternate black pepper germplasm conservation center at CHES, Chettalli.

Organic production package

Organic production package involving 10 kg FYM + 500g neem cake + 500g ash+ 2kg vermicompost with biofertilizers – *Azospirillum* and P solubilizing bacteria (20g) and *Trichoderma* (50g) and *Pseudomonas* (IISR 6) (50g) per vine and spray of 1% Bordeaux mixture (BM) and neem oil (5mL L^{-1} of water) for disease and pest control has been developed, tested and demonstrated. Results showed that organic management system yielded on par with integrated system, whereas oleoresin content was found to be superior under organic system.

Black pepper- tree species interaction

The extracts of trees like garuga, erythrina, gliricidia, ailanthus, jack and silver oak, the most commonly used support trees of black pepper at 100% concentration was applied to bush pepper @100mL per plant twice every month. Observations on growth parameters taken after 8 months of application revealed little or no significant differences among the treatments including control. Available nutrient levels including organic C were markedly high (5.4-6.0%) in all the treatments. Dehydrogenase level was lowest in the control and silver oak, but markedly higher in soils applied with other tree extracts. Microbial biomass C was lowest with silver oak and ailanthus extracts but greatest with garuga extract.

Microbial consortium for growth promotion

A consortium of three rhizobacterial isolates (BRB3, BRB13 and BRB23) markedly promoted the growth of black pepper. However, the PGPR applied in combination with various rates of chemical fertilizers differed in their effects on growth parameters. Shoot weight (fresh) was highest with 75%N + 100%P + 100%K + BRB3 + BRB13 + BRB23, while greatest root weight (fresh) and tallest plants were registered in the treatment 100%N + 100%P + 75%K + BRB3 + BRB23. Based on a series of experiments, microbial formulation involving a consortium of PGPR *viz.*, IISR Biomix was developed for licensing and commercialization.

Drought tolerance

Fifty germplasm accessions were screened for drought tolerance and the accession 6707 was identified as relatively tolerant based on its high relative water content (RWC) and low membrane leakage (ML) values even at 8.0-8.5% soil moisture content.

New chemicals against foot rot

The antioomycete activity of RIL-070/FI (72WP) and Ergon 44.3% (w/w) were tested against

different developmental stages of *Phytophthora capsici* and *Colletotrichum gloeosporioides* infecting black pepper. RIL-070/FI (72WP) when tested *in vitro* showed 100% inhibition against mycelia at 50ppm, sporulation at 100ppm and zoospore germination at 200ppm concentrations. The average ED50 value of RIL-070/FI (72WP) against *P. capsici* was 30ppm. Ergon 44.3% (w/w) showed 100% inhibition of mycelial growth and sporulation at 6000ppm with an ED50 value of 210.6ppm for mycelia inhibition and 1480.4ppm for sporulation inhibition. Inhibition of zoospore germination was 100% at 1000ppm.

Evaluation of new chemicals against *Radopholus similis*

Nematicidal activity of eight chemicals *viz*., Fipronil, Thiamethoxan, Acephate, Cartap hydrochloride, Quinalphos, Flubendamide, Carbosulfan and Chloropyriphos was evaluated against *R. similis* in the green house. Out of these, 5 chemicals *viz*., Fipronil, Thiamethoxan, Cartap hydrochloride, and Carbosulfan showed good nematicidal activity.

Greenhouse evaluation of actinomycetes against *P. capsici*

Nine actinomycetes were evaluated under green house conditions for disease suppression and growth promotion. The results revealed the efficacy of 3 isolates (Act5, Act2, and Act9) in reducing foot rot incidence due to *P. capsici* under challenge inoculation. The potential isolates were identified as *Kitasatospora setae* (Act2), *Streptomyces* sp. (Act5) and *S. tauricus* (Act9).

Development of liquid formulation for biological control agents

Mature conidial suspension in sterile deionized water was a promising medium for long term storage and preservation of viable conidia of *Trichoderma* with consistent biocontrol potency. An encapsulated bead formulation of *Trichoderma harzianum* and *Pochonia chlamydosporia* was also formulated.

Characterization of *Phytophthora* isolates using multi-gene approach

A multi-gene analysis was done for the characterization of *Phytophthora* isolates from black pepper. Nine loci were selected *viz*., 28S Ribosomal DNA, 60S Ribosomal protein L10, Beta-tubulin, Elongation factor 1α, Enolase, Heat shock protein 90, TigA gene fusion protein, Mitochondrial genome region between gene COX2 and gene COX1 and Ras-related protein (Ypt1) gene. All the loci were amplified using the corresponding primers, which revealed 2 diverse groups *viz*., where Group 1 showed similarity to *P. capsici* and Group II showed similarity to both *P. capsici* and *P. tropicalis*.

Identification of virulence-associated *Phytophthora* gene

Gene expression studies using qPCR undertaken in *P. colubrinum* challenge inoculated with *Phytophthora* revealed highest level of expression of polygalacturonase inhibitor protein (PGIP), indicating its importance in disease resistance.

Evaluation of *Trichoderma* isolates

Fifteen different isolates of *Trichoderma* isolated as efficient for various crops were tested against *P. capsici.* PhytoFuRa10 was found to be highly promising with less than 10% disease incidence followed by PhytoFuRa8 , PhytoFuRa11, and PhytoFuRa13 with 10- 30% disease incidence suggesting the plasticity of isolates.

Potential endophytic fungi

Endophytic fungi were evaluated for its biocontrol potential against *P. capsici in vitro*. The inhibition ranged from 70-78% and the isolates were identified as species of *Diaporthe, Phomopsis, Annulohypoxylon nitens, Daldinia eschscholzii, Fusarium proliferatum, Fusarium moniliformae* and *Ceriporia lacerata.*

Radopholus similis diversity

Sixteen isolates of *R. similis* collected from different parts of Kerala were studied for their genetic diversity through ITS-PCR and sequencing. Phylogeographic studies using ITS sequences of *R. similis* isolates from different states of India revealed extensive genetic diversity in African and Indian populations.

Phenyl propanoids vis-à-vis *Radopholus*-black pepper interaction

Quantitative assays to estimate phenyl propanoids indicated that the levels of anthocyanidins and total phenols were significantly high in HP39, the nematode resistant hybrid, compared to the susceptible Sreekara variety. Histochemical studies proved that lignification was significantly high in HP39 roots compared to Sreekara.

Studies on endophytic bacteria

Endophytic bacteria such as spontaneous rifampicin resistant *Bacillus megaterium* BP17R and *Pseudomonas putida* BP25R were characterized by Biolog, biochemical and antibiotic sensitivity assays. Reaction of the above 2 bacteria to 17 antibiotics indicated that *P. putida* BP25 was resistant to several antibiotics while *B. megaterium* BP17 was resistant only to rifampicin. The whole metabolome extracted from BP17R and BP25R on assaying against *P. capsici, P. tropicalis, P. myriotylum, Rhizoctonia, Fusarium, R. solanacearum* and nematodes by *in vitro* bioassays did not show any activity against any of the tested pathogens, including nematodes. However, at a concentration of 20mg mL^{-1} these extracts inhibited germination of *P. capsici* zoospores.

Technology for managing anthracnose disease in nursery

Pre-planting treatment of 3 node cuttings from the runner shoots with the combination of carbendazim + mancozeb (0.1%) was found to be superior in delaying disease initiation and development compared to other treatments and was validated and recommended for the management of the disease in the nursery.

Real-time PCR and LAMP for detection of viruses

A SYBR Green based quantitative real time polymerase chain reaction (qPCR) for Piper yellow mottle virus (PYMoV) and reverse transcription (RT) qPCR for Cucumber mosaic virus (CMV) infecting black pepper was developed for sensitive and specific detection and quantification of these viruses. qPCR and RT-qPCR were more sensitive than conventional PCR and RT-PCR in the detection of viruses. The method was validated using field samples collected from different regions.

A quick detection assay based on loop mediated isothermal amplification (LAMP) was developed for detection of PYMoV and reverse transcriptase (RT) LAMP for detection of CMV infecting black pepper. The assay successfully detected both the viruses in infected plants while no cross-reactions were seen with healthy plants. Detection limit of LAMP was up to 100 times higher than conventional PCR and up to 100 times less sensitive than real time PCR. The optimized LAMP and RT-LAMP were validated by testing field samples of black pepper collected from different regions.





CARDAMOM

Genetic resources

A total of 592 accessions have been maintained in the National Active Germplasm Site (NAGS) at Appangala. Five accessions including high biomass type, deep green capsules and drought tolerant types were collected from Sultania region of Megamalai area. Appangala-1 variety was registered with Protection of Plant Variety & Farmers Rights Authority, New Delhi.

Drought tolerance

Twelve elite lines with 3 checks were evaluated for drought tolerance under field condition. Significant variations were recorded for growth with reduction in yield characters under stress. Two genotypes with bold capsule and early maturity maintained yield under moisture stress.

Mycelial compatibility among isolates of *C. gloeosporioides*

Mycelial compatibility studies among the selected isolates of *C. gloeosporioides* (3 each from Kerala and Tamil Nadu and 8 isolates from Karnataka) revealed that majority of the isolates showed incompatible reaction when isolates from different geographical locations were paired. Isolates from same geographical region exhibited compatible reaction.

Characterization of endophytes

Endophytic fungi and bacteria were isolated from three ecotypes *viz., Malabar, Mysore* and *Vazhukka*. Isolations made from different plant parts including leaves, petioles, pseudostem, roots and rhizomes of *Amomum* sp. and *Alpinia* sp. yielded 50 fungal isolates and 5 bacterial isolates. Among the plant parts, the isolates had more preference for pseudostem followed by rhizomes.

IPM for leaf spot disease

Evaluation of fungicides, a neem based product and an isolate of *Trichoderma harzianum* revealed that spraying the combination product of carbendazim + mancozeb (0.1%) in combination with soil application of *T. harzianum* was promising in managing leaf spot disease under nursery conditions.

Diversity of rhizome- root rot pathogens

Surveys were repeated in Wayanad and Idukki districts of Kerala, Hassan and Kodagu districts of Karnataka to study the seasonal variation of rhizome and root rot diseases. Eighty fungal isolates were isolated from rhizome and root rot disease affected samples. Among the isolates, *Rhizoctonia solani, Pythium vexans* and *Fusarium* species were found to be dominant. Artificial inoculation studies proved that among the different fungi isolated, *R. solani, P. vexans* and *F. oxysporum* were pathogenic and *F. oxysporum* was found to be the predominant species. *In vitro* screening of respective *Trichoderma* spp. against the most virulent *F. oxysporum* isolates from Kerala, Karnataka and Tamil Nadu led to the identification of WYD T6, RT 7B and RT 2A, respectively as the most effective isolates.

RT-PCR and RT-LAMP for detection of viruses

A SYBR Green based real-time quantitative RT-PCR (qRT-PCR) for detection of Cardamom mosaic virus (CdMV) and Banana bract mosaic virus (BBrMV) was developed. RT-qPCR was more sensitive than conventional RT-PCR. Reverse transcriptase (RT) LAMP method was also developed for quick and sensitive detection of BBrMV infecting cardamom. Detection limit of RT-LAMP was higher than conventional RT-PCR. The optimized techniques were validated by testing field samples of cardamom collected from different regions.

Screening for thrips resistance

Two hundred and ninety six accessions/ lines were field screened against thrips during the year. Fifty three accessions recorded capsule damage below 20%. IC 349441 recorded the lowest capsule damage of 3%. Five Accessions recorded below 10% capsule damage. Nine accessions recorded more than 70% capsule damage with IC 349520 exhibiting the highest damage of 96.2%. The accessions with less than 10% capsule damage belonged to *Malabar* type except IC 349416 which belonged to *Vazhukka*. All the 9 highly susceptible accessions belonged to either *Mysore* or *Vazhukka* types.

Bacterial endosymbiont and entomopathogen

The endosymbiont *Wolbachia* was identified in thrips populations collected from Kodagu (Karnataka), Wayanad, Palakkad, Idukki (Kerala), Yercaud, Ooty and Dindigul districts (Tamil Nadu). Both larvae and adults (male and female) were observed to harbour the endosymbiont.

Management of thrips

Ten new insecticides and organic prod-

ucts such as neem soap, Spinosad, Vertemec, Thiamethoxam, Thiacloprid, Imidacloprid, L-cyhalothrin, Zolone, Fipronil and Quinalphos were evaluated in the field for the management of thrips. Among the treatments, Fipronil (1.0mL L^{-1}) was the most effective and was on par with Imidacloprid (0.5mL L^{-1}), Quinalphos (2mL L^{-1}), Thiacloprid (0.5mL L^{-1}), and Thiamethoxam (0.3mL L^{-1}). Neem soap was not effective and was at par with control.

TURMERIC

Genetic resources

One thousand three hundred and forty two *Curcuma* accessions are being maintained. Germplasm conservatory was enriched with 92 accessions received from RAU, Dholi, Bihar and TNAU, Coimbatore, Tamil Nadu as part of NAGS. Promising nematode tolerant accessions *viz.*, Acc. 48 and Acc. 79 have been shortlisted for yield. Six novel polymorphic SSR markers were isolated by selective hybridization method using 3' biotinylated microsatellite probe mix [(ACT)12, (AAAC)6, (ACCT)6] for identification of different accessions.

Source-sink relationship

Varieties IISR Prathibha, IISR Alleppey Supreme and Suguna were sampled at monthly intervals starting from 50 days after planting (DAP). All the varieties were on par with regard to leaf area and dry weight. Maximum levels of endogenous IAA and zeatin riboside levels were recorded at 110 DAP which was just prior to rapid rhizome bulking. Rapid starch accumulation (rhizome bulking) was seen after 120 DAP. The level of photosynthesis was observed to be lowest in Suguna at 130 DAP.

Micronutrients for yield and quality

Based on the field studies on effect of Zn and B on the quality of IISR Prathibha for 3 years, soil application of Zn up to 10kg ha^{-1} or foliar spraying of $ZnSO_4$ (0.25%) and Borax (0.2%) twice (60 and 90 DAP) is recommended for high yield and quality, especially in soils deficient in Zn and B.

Organic production package

Organic package consisting of 20t FYM ha^{-1} + 2t ha^{-1} neem cake + 1t ha^{-1} ash + 4t ha^{-1} vermicompost, *Azospirillum* and P solubilising bacteria (20g bed^{-1}) and PGPR (GRB35) as seed treatment and as drench at 45 and 90 DAP has been standardized. Application of BM spray (1%) to contain foliar diseases and neem oil (5ml L^{-1} of water) in combination with the cultural control is recommended for shoot borer management. Results showed that organic management system yielded on par with integrated system, while curcumin content was greater under organic system.

Chemoprofiling of varieties

Essential oil profile of rhizomes of 7 varieties indicated turmerone (5.6-25.8%), ar- turmerone (3.5-20.8%) and curlone (5.4-15.6%) as the chief components. Among the varieties, ar-turmerone was low in Sobha (3.5%). Turmerone was 20-25% in Rajendra Sonia, Sugandham, Narendra Haldi and Co-1, 5-7% in Sobha and Sona and 13% in Varna. With regard to curlone, Varna, Rajendra Sonia, Sobha and Sona formed a group with 5.0-6.8% and Sugandham, Narendra Haldi and Co-1 formed another group with 12.5-15.6%.

Biochemical characterization for resistance to shoot borer

In mature leaves of resistant accessions, carbohydrates and proteins ranged from 7.56-19.71 and 1.08-7.75mg 100 mg^{-1} of dried leaves, respectively. The lignin content ranged from 26.4-48.7%. In mature leaves of susceptible accessions; carbohydrates and proteins ranged from 8.78-12.76 and 2.82-11.10mg 100 mg^{-1} of dried leaves. The lignin contents ranged from 23.9-39.1%. The lignin content in immature shoots of both resistant and susceptible accessions was identical and ranged from 43.0-44.0%. The fiber content in immature shoots of resistant and susceptible accessions ranged from 24.3-30.3% and 25.7-37.0%, respectively.





Evaluation of entomopathogenic nematodes (EPNs)

Among the EPNs tested against shoot borer, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* treated plants showed least shoot damage (26.1 and 26.6%, respectively).

GINGER

Genetic resources

Six hundred and sixty eight accessions have been conserved under field genebank. The RAU, Dholi, Bihar deposited 36 accessions to NAGS. Promising nematode tolerant accession (Acc. 219) has been shortlisted for yield.

Induction of variability through induced mutation

The rhizome buds of 3 varieties (Mahima, Varada and Rejatha) were subjected to γ irradiation at different doses. Based on probit analysis for mortality percent, LD50 for these varieties was derived. One hundred and twenty eight mutants (M1V7 and M1V6) were subjected to preliminary screening for soft rot disease caused by *Pythium myriotylum* and 35 mutants which escaped infection were short listed.

Source-sink relationship

The varieties IISR Varada, IISR Rejatha and IISR Mahima were sampled at monthly intervals starting from 50 DAP and Varada accumulated more leaf area and dry weight. Endogenous IAA and zeatin riboside levels were greatest in rhizomes at 80 DAP. Rapid starch accumulation (rhizome bulking) was seen after 100 DAP and all the varieties showed similar levels of photosynthesis at 130 DAP.

Organic production package

Organic package consisting of 20t ha^{-1} FYM + 2t ha^{-1} neem cake + 1t ha^{-1} ash + 4t ha^{-1} vermicompost, *Azospirillum* and P solubilising bacteria (20g bed^{-1}) and PGPR (GRB35) as seed treatment and as drench at 45 and 90 DAP has been standardized. BM spray (1%) to contain foliar diseases and neem oil (5mL L^{-1} of water) in combination with the cultural control is recommended for shoot borer. Results showed that organic management system yielded on par with integrated system, while quality was superior under organic system.

Chemoprofiling of varieties

Volatile oil profile of 7 ginger varieties, *viz*., Varada, Mahima, Rejatha, Suprabha, Surabhi, Himgiri and Rio de Janeiro was determined. The major constituents were zingiberene (20-23%), farnesene (9-12%), β- sesquiphellandrene (11-12%), ar curcumene (8.9-10.3%), bisabolene (1.9-2.6%), and β- phellandrene (2.8-3.2%). Zingiberene was low in Rio-de Janeiro with 16.4% whereas ar-curcumene was low in Varada (5.7%).

Ginger transcriptomics

A total of 56,090,429 and 42,386,900 PE reads were obtained after quality filtering for *C. amada* and *Z. officinale*, respectively. A total of 45046 and 65536 assembled transcript sequences were obtained from both the species. The functions of the unigenes, from GO annotation, cover a diverse set of molecular functions and biological processes, among which we identified a large number of genes associated with resistance to stresses and response to biotic stimuli. Large scale expression profiling showed that many of the disease resistance related genes were expressed more in *C. amada*. Comparative analysis also identified genes belonging to different pathways of plant defense against biotic stresses that are differentially expressed in either ginger or mango ginger.

Antimicrobial properties of *C. amada*

Essential oil extracted from the dried rhizomes of *C. amada* exhibited maximum antimicrobial activity on *P. myriotylum* and *R. solan-*

acearum. β-myrcene and β-pinene were the major components in essential oil.

Phage therapy against *R. solanacearum*

Phages were isolated from ginger rhizosphere soil from Wayanad, using *R. solanacearum* isolate from Wayanad as the host. The phage was found to be highly host specific showing infection only towards *R. solanacearum* isolates collected from the Wayanad and not towards any other isolate.

Biochemical characterization for resistance to shoot borer

Epicuticular wax, lignin and fiber contents in tender leaves and lignin and fiber contents of tender shoots were determined in moderately resistant and susceptible accessions. In mature leaves of resistant accessions, carbohydrates and proteins ranged from 6.22-13.05 and 1.47-4.61 mg 100mg^{-1} of dried leaves, respectively. The lignin content ranged from 10.05-18.94%. In mature leaves of susceptible accessions, carbohydrates and proteins ranged from 6.86-16.31 and 1.28-2.72mg 100mg^{-1} of dried leaves. In mature shoots of resistant accessions, the fiber and lignin contents ranged from 24.6-34.3% and 8.59-17.6%, respectively. In mature shoots of susceptible accessions, the fiber and lignin contents ranged from 19.3-27.7% and 17.09-17.6%, respectively.

EPNs and its mass production

Among EPNs tested against shoot borer in the green house, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* treated plants showed least shoot damage (15.8 and 16.6%, respectively). Multiplication of 4 EPNs *viz., Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius* sp. (IISR-EPN 08) and *O. gingeri* in 3 modified Wouts medium (MWM) was tested. Among the various media, all tested EPNs were able to multiply in MWM-I. However, maximum numbers of *O. gingeri* were produced in MWM-II.

TREE SPICES

Nutmeg

Surveys were conducted in farmers' fields in adjoining areas of Pala and Thodupuzha (Kerala State) and 6 nutmeg accessions were collected. They are *Cheripurathu* (entire mace), *Madukkakuzhi* (bisexual, producing more of clustered fruits), 2 yellow mace types, *Kinattukara* and *Kochukudi* (high yielding types).

Garcinia

Biochemical properties of garcinia seed fat of 4 species were standardized. Palmitic, stearic, elaidic, oleic, linoleic, arachidic and eicosenoic acids, which are important constituents of fatty acids were detected using GCMS with equal number of saturated and unsaturated fatty acids. ITS region for 9 species of garcinia were sequenced and deposited in the NCBI data base (JX472233-JX472241).

PROCESSING AND VALUE ADDITION

Flavouring and medicinal property of cryogenically ground sample

Cryo ground black pepper Panniyur-1 yielded high oleoresin (9.5%) compared to ambient ground (8.0%). Freshly ground sample gave high oil and comparable oleoresin. Piperine content did not show any variation. Major sesquiterpenes like β–caryophyllene was 27.7% in cryo-ground sample compared to 26.0% in ambient.

Turmeric curing and drying

Experiments on cooking of turmeric at three varying steam pressures (0, 0.5 and 1kg cm^{-2}) corresponding to steam temperatures of 100, 112 and 121^{o}C for 4 different cooking durations (5, 10, 15 and 20 min) were performed in a pilot-scale to determine the time required for drying and to evaluate the quality. Studies indicated that irre-

spective of the treatment combinations, the drying time required for bringing down the moisture content to <10% was 7 days. Quality analysis of the dry turmeric samples indicated reduction in curcumin content from 4.66-4.44% when the curing time was increased from 30 min to 90 min in case of improved curing method and from 4.95-4.62% when the curing time was increased from 30 min to 90 min in case of water boiling method.

Food extrudates from spices

Extrusion cooking is a high-temperature short-time (HTST) processing technology wherein the cassava flour was mixed with cardamom powder in the ratio of 96:4, and conditioned at 4°C for 15 days. The study indicated that extrusion cooking of cassava flour blended with cardamom powder produced extrudates which had an overall sensory acceptability score of 6.3.

BIOINFORMATICS

Phytophthora genome sequencing and annotation

The complete genome of two *Phytophthora* isolates (05-06 and 98-93) infesting black pepper was sequenced using Illumina/Roche 454 platforms. The cross-platform sequence data was *de novo* assembled and annotated structurally and functionally to curate all possible gene by gene information. Whole genome alignment with the reference genome revealed that 05-06 is 95.35% similar while 98-93 is 87.90% similar to the reference genome. Conserved domain search to identify the protein families present in exonic regions of whole genome sequences of the 2 different isolates of *Phytophthora* sp. infecting black pepper (05-06 and 98-93) has also been carried out along with Blast2GO analysis.

Species and evolutionary tree estimation of sequenced *Phytophthora* isolates

The evolutionary history of the *Phytophthora* genomes sequenced was traced out using information from multilocus gene trees for 4 mitochondrial and 10 nuclear markers from 6 closely related species of Clade 2. The concatenation-based multispecies coalescent approach using Bayesian, maximum parsimony and maximum likelihood methods was able to estimate a moderately supported species tree showing a close relationship among 05-06 with *P. capsici* and 98-93 with *P. tropicalis*.

New databases developed

- Plant Virus Database: It provides a central source of information about all the plant viruses in India.
- *Phytophthora* Genome Database: Sequence information of hybrid assemblies of 2 isolates of *Phytophthora* infesting black pepper was incorporated to the existing database.

EXTENSION AND TRAINING

Training programme on spices production technology was conducted for officers from Horticulture Research and Training Centre (HRTC), Jhansi, Department of Horticulture and Food Processing, Uttar Pradesh and for 25 trainees from Andhra Pradesh and Karnataka. A training workshop on production management, on farm processing and post harvest technology was organized in collaboration with the Spices Board at Itanagar, Arunachal Pradesh during 18-20 February 2013 for 50 farmers and 8 officers. An on demand training programme on black pepper production management was organized for 9 estate managers of Harrison Malayalam Plantations Pvt. Ltd.

Impact assessment surveys

The study in Guntur district in Andhra Pradesh revealed that the turmeric variety IISR Prathibha has spread to about 250ha as first crop through farmer to farmer lateral spread and ex-

change of planting material. Even though dominant area is under traditional cultivars like Dugirala, Kadappa and Tekurpet, the IISR Prathibha cultivators reported an average yield of 35t ha^{-1} whereas the average yield of local cultivars was around 20t ha^{-1}. In a farm at Vellamunda, Wayanad IISR Prathibha was grown in 16 acres (Bucca farms). Success story of IISR Prathibha was highlighted on the ICAR homepage.

Studies on nutmeg showed that IISR variety Viswasree has been introduced as a dominant high yielding intercrop in coconut gardens. Popularisation of the line started since 2000 and the present standing crop is at full bearing stage of around 12 years. The reported average yield under proper shade, canopy management and assured irrigation is 1500 nuts $tree^{-1}$ yr^{-1}.

Soil based nutrient management plans for agro-ecosystems of Kerala

Out of 17069 soil samples received from 74 Panchayats of Kozhikode district, 10110 soil samples representing 47 Panchayats have been analyzed for pH, EC, major- and secondary- and micro-nutrients. Results of 9200 soil samples representing 43 Panchayats have been uploaded into www.keralasoilfertility.net.

Technology mission for pepper in Wayanad

About 750 soil samples from Thirunelli Panchayat of Wayanad district were analyzed for major, secondary and micro nutrients and results with site specific recommendations was passed on to the farmers. Pamphlets were prepared in Malayalam on composting, use of pesticides, Biocontrol of pest and diseases and distributed to farmers. Twenty five farmer's plots were selected in three Panchayats and FLDs on varieties and technologies to rejuvenate yellowing of black pepper is initiated and the inputs like planting material, lime, organic manures, neem cake, micro nutrient mixtures and bio agents were supplied.

Mass Media Support for Sharing Agro-Information

- Media visit has been arranged for 10 journalists (Print/TV/Radio) at the turmeric plot of Muhammed Busthani, Wayanad.
- Five journalists participated in the visit to IISR Viswashree farmer's field at Karuvarakkundu, Malappuram
- More than 20 Success Stories and 40 news items were published in various Malayalam and English newspapers/magazines and news portals
- Seven capsules (interviews and success stories) broadcasted through AIR Madikeri

Kisan Mela

Participatory farmer's Mela was organized at Cardamom Research Centre, Appangala for showcasing technologies during 20-22 December, 2012. The exhibition was inaugurated by Dr. S. Ayyappan, DG, ICAR and held for 3 days. The Farmers Scientist interactive meet was held on 21 December under the chairmanship of Dr. M.R. Sudarshan, Director (Marketing), Spices Board and was inaugurated by the Hon'ble speaker Karnataka Assembly Mr. K.G. Bopaiah. Six innovative farmers were felicitated. Various government and self help groups showcased their products/ technologies.





INSTITUTE TECHNOLOGY MANAGEMENT UNIT

- The unit has facilitated non-exclusive license agreements in turmeric and ginger varieties, IISR Prathibha and IISR Varada, respectively with National Horticultural Research and Development Foundation (NHRDF), and Katra Phyto Chem. Pvt. Ltd, Bangalore for





IISR Prathiba and with Mr. Tom C Antony, Cheripurathu Nursery, Kottayam for nutmeg variety IISR Viswashree.

- Six formulations of crop specific micronutrient mixtures developed are in the process of commercialization and patenting. Seed dressing technology for seed spices, microbial consortium for black pepper, PGPR talc formulations for ginger and diagnostics for virus detection in black pepper are also in the process of commercialization through National Research Development Corporation (NRDC), New Delhi.
- The invention entitled "Bacterial fermentation technology for production of high quality off-odour-free white pepper from matured green pepper (*Piper nigrum L.*)" was filed for patent (Application No.3433/CHE/2011 A; dated 20/04/12).
- A novel technology for delivery of PGPR is in process for patent filing and commercialization.
- Released variety of cardamom, Appangala-1 was approved for registration as extant variety by the PPV&FRA (Registration No. 134/2012). Ten other varieties are in the process of approval by PPV & FRA.
- A book entitled "IPR: Current Scenario in Spices" was published.

KRISHI VIGYAN KENDRA

About 141 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted and 5157 trainees were benefitted out of the programmes. Fourteen Front Line Demonstrations and 10 On Farm Trials on technology assessment and refinement were carried out. Besides, 605 plant-animal clinic consultancy services, 47400 vaccinations of poultry birds and animals and 5 animal health campaigns were conducted. Participatory seed production on high yielding varieties of ginger and turmeric was also taken up in 10 farmers plots. About 30 Short Message Service (SMS) on latest updates in agriculture and allied fields were sent to 742 farmers and 100 extension functionaries. Also conducted 9 seminars, participated in 8 Kisan Mela cum exhibitions, broadcasted 2 radio talks and 6 study tours for farmers. The KVK was also conferred with the best KVK Award 2011 for Zone VIII for its outstanding contributions which includes a certificate, citation, and cash prize of ₹ 4 lakhs.

HUMAN RESOURCE DEVELOPMENT

Trainings conducted

- Cheminformatics- Tools and Applications from 19 to 22 February 2013.
- Next Generation Sequencing- data analysis and annotation from 12 to 16 March 2013.
- One month summer training on biochemistry, biotechnology and bioinformatics for eight M.Sc. students from 8 May to 6 June 2012.
- One student completed post M.Sc. training and 5 students were awarded with Ph.D.

INTRODUCTION

History

Intensive research on spices in the country was initiated with the establishment of a Regional Station of Central Plantation Crops Research Institute (CPCRI) at Calicut, Kerala, during 1975, by the Indian Council of Agricultural Research (ICAR). This Regional Station was upgraded as National Research Centre for Spices (NRCS) in 1986 by merging with it the Cardamom Research Centre of CPCRI at Appangala, Madikeri, Karnataka. The NRCS was further elevated to the present Indian Institute of Spices Research (IISR) during 1995.

Location

The laboratories and administrative offices of the institute are located at Chelavoor (50 m above MSL), 11 km from Calicut (Kozhikode), Kozhikode District, Kerala, on the Calicut- Kollegal road (NH 212), in an area of 14.3 ha. The research farm is located 51 km North East of Calicut at Peruvannamuzhi (60 m above MSL), on the Peruvannamuzhi-Poozhithode road in Kozhikode District, in an area of 94.08 ha. The Cardamom Research Centre, Appangala (920 m above MSL) is located at Appangala, Kodagu District, Karnataka, on the Madikeri-Bhagamandala road, 8 km from Madikeri, in an area of 17.4 ha.

Mandate

- To extend services and technologies to conserve genetic resources of spices as well as soil, water and air of spices agroecosystems.
- To develop high yielding and high quality spice varieties and sustainable production and protection systems using traditional and non-traditional techniques and novel biotechnological approaches.
- To develop post harvest technologies of spices with emphasis on product development and product diversification for domestic and export purposes.
- To act as a centre for training and technology upgradation of spices and to coordinate national research projects.
- To monitor the adoption of new and existing technologies to make sure that research is targeted to the needs of the farming community.
- To serve as a national centre for storage, retrieval and dissemination of technological information on spices.

The spice crops on which research is being conducted at the institute include black pepper (*Piper nigrum* Linn.), cardamom (*Elettaria cardamomum* Maton), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Linn.), cinnamon (*Cinnamomum verum* J. Presl.), cassia (*C. cassia* Nees ex Blume), clove (*Syzygium aromaticum* (L.) Merrill & Perry), nutmeg (*Myristica fragrans* Houtt.), allspice (*Pimenta dioica* (L.) Merrill & Perry), Garcinia (*Garcinia gummi-gutta* (L.) N. Robson and *G. indica* Choisy) and vanilla (*Vanilla planifolia* Jacks. ex Andrews).

Organization

The Director is the administrative head of the institute. The Institute Management Committee, Research Advisory Committee and Institute Research Committee assist the Director in matters relating to management and research activities of the institute. Research on various aspects of the mandate crops is conducted in 3 divisions, namely, Division of Crop Improvement and Biotechnol-



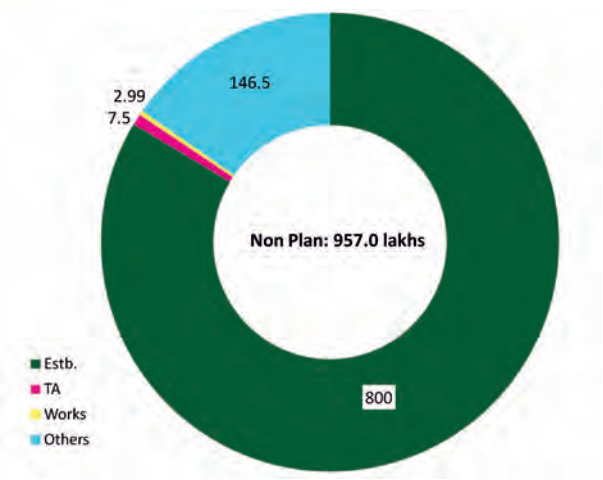


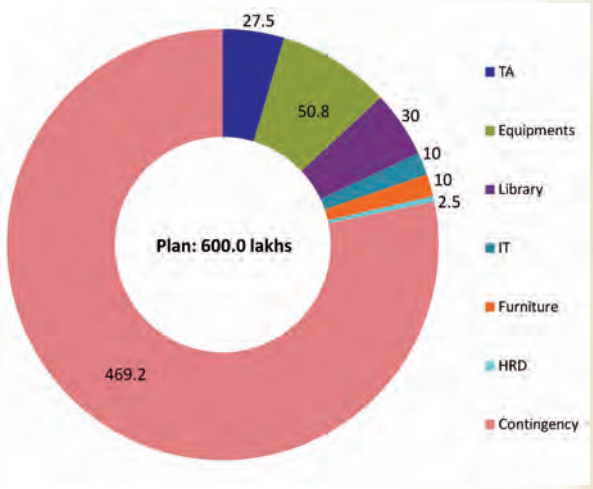
ogy, Division of Crop Production and Post Harvest Technology and Division of Crop Protection and a Social Sciences Section. The other facilities available at the institute include Agricultural Technology Information Centre, Agricultural Knowledge Management Unit, Institute Technology Management Unit (ITMU), Hindi Cell, Bioinformatics Centre and Krishi Vigyan Kendra. The institute also functions as the headquarters for the All India Coordinated Research Project on Spices (AICRPS), and Indian Society for Spices (ISS). An outreach project on Phytophthora, Fusarium and Ralstonia diseases of horticultural and field crops was sanctioned in the XI plan (2007-12) with IISR, Calicut as the lead centre and 17 coordinating centres at different ICAR institutes/ SAUs across India. The institute has also linkages with several universities, research institutes, and developmental agencies for collaborative research and developmental activities in spices.

Budget: The total budget of the institute was ₹ 1557 lakhs during the year, which included ₹ 600.00 lakhs (including OPR on PhytoFuRa) under Plan and ₹ 957 lakhs under Non Plan.

Resource generation: Institute earned a total of ₹ 23.5 lakhs through sale of planting materials, biocontrol agents, training, publications and consultancy services.

Staff: The institute has a sanctioned strength of 44 scientific, 24 administrative, 31 technical and 33 supporting staff, of which 32, 20, 28 and 33 of scientific, administrative, technical and supporting staff, respectively are in position. The KVK has a sanctioned strength of 2 administrative, 12 technical and 2 supporting staff.





Staff Position of the Institute

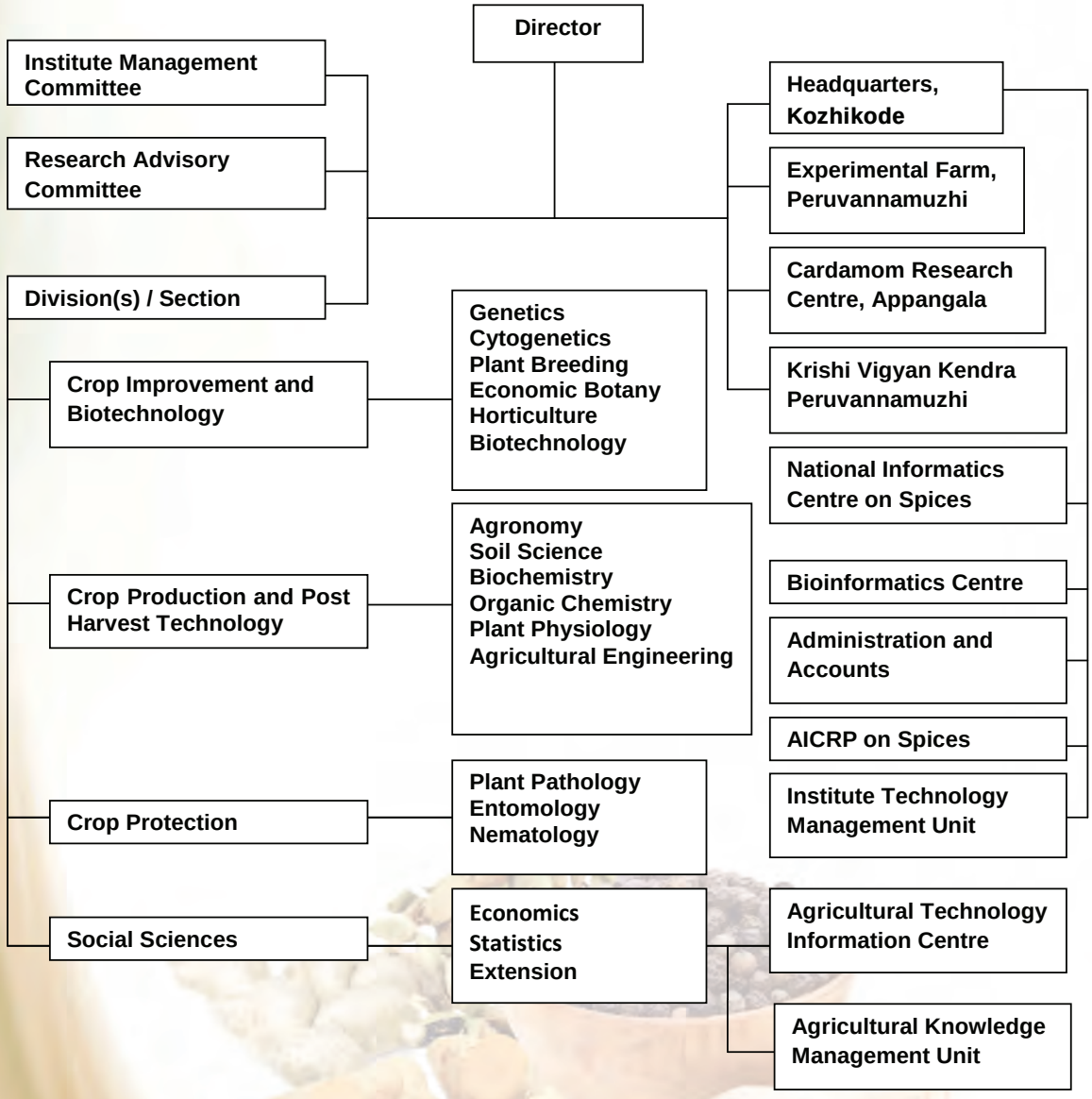
Category	Sanctioned	In position			Total	Vacant
		Kozhikode	Peruvannamuzhi	Appangala		
Scientist	44	27	1	4	32	12
Technical	31	15	9	4	28	3
Administration	24	18	-	2	20	4
Supporting	33	10	7	16	33	-
Total	132	70	17	26	113	19

Staff position of KVK

Category	Sanctioned	In position	Total	Vacant
Technical	12	11	11	1
Administration	2	1	1	1
Supporting	2	2	2	-
Total	16	14	14	2







Organizational chart of Indian Institute of Spices Research, Kozhikode

Deep trenches for preventing wild animal's entry at Peruvannamuzhi farm

Soil and water conservation measures undertaken at Chelavoor campus

Nursery complex for planting material production at Chelavoor campus

PAST ACHIEVEMENTS





Black pepper: Germplasm collections obtained over the years through explorations are being maintained at IISR as well as in other alternate sites *viz.*, Appangala and Kidu of Karnataka for developing improved varieties for yield, quality, abiotic and biotic stresses. The genetic stock has lead to release of improved varieties such as Sreekara, Subhakara, Panchami and Pournami, IISR PLD-2, IISR Thevam, IISR Girimunda, IISR Malabar Excel and IISR Shakthi. Front-line demonstration programme was undertaken using the released varieties in the farmers' field. Some of the unique germplasm have been registered with NBPGR at New Delhi. Two accessions, INGR 8099- *Piper thomsonii* (IC 398863) - for its unique character for sex change and INGR 8100- *Piper nigrum* (IC 563950) – A novel spike variant with proliferating spikes, were registered with NBPGR for their unique characters. Endangered species *viz*. *P. barberi* and *P. hapnium* were located and collected from Sabari hills. Microsatellites developed for *Piper* sp. were successfully used to detect polymorphism in black pepper cultivars. Assembly and functional annotation of sequences derived from the transcriptome of *P. colubriunum* and *P. nigrum* helped in the identification of many genes involved in secondary metabolism. *P. colubriunum* and *P. nigrum* transcripts showed maximum hit with *Vitis vinifera* (wine grape) sequences, followed by *Populus trichocarpa* (Poplar) sequences indicating closer relationship of magnoliids (order to which *Piper* belong to) with eudicots. Seedlings of *P. colubrinum* on screening with *P. capsici* showed segregation of the resistance character, 21 plants being resistant to *Phytophthora*, 2 plants *susceptible* and the rest showing moderate resistance.

Putative transgenic black pepper plants with osmotin gene conferring resistance to drought and *P. capsici* has been developed. *In vitro* and *in vivo* propagation methods were standardized. Plantlets developed through micropropagation were established in farmers' field in Kerala and Karnataka. Portion of gene conferring resistance against *P. capsici* was isolated by targeted gene amplification using degenerate primers from *P. colubrinum*. The spacing, nutrient and water requirements were standardized for different soil types of pepper growing regions. Irrigating pepper vines once in a fortnight from March to May months at the rate of 50L vine^{-1} enhanced yield significantly. Vines which received 200-300µmoles m^{-2} sec^{-1} produced 3.4kg vine^{-1} and those which received around 100µmoles m^{-2} sec^{-1} produced 1.8kg vine^{-1} under Madikeri conditions. High production technologies and mixed cropping systems were developed for increasing productivity. Among different forms of K, water-soluble and available K had significant positive correlation with berry yield, oleoresin and piperine. Organic production technology for black pepper has been standardized. Crops such as ginger, tapioca, coleus, amorphophallus and hybrid napier were found suitable for intercropping in black pepper gardens that are more than 15 years old. Intercropping medicinal plants (*Vetiveria zizanoids* and *Alpinia calcarata*) in juvenile black pepper garden was found to be profitable with a B:C ratio of 2.3. Cost effective method for production of disease-free





rooted cuttings was developed. A machine was fabricated in collaboration with CIAE, Coimbatore centre which is capable of mixing, pulverizing, sieving, and filling of potting ingredients in poly bags at desired quantity. Mathematical models for optimum climatic factors for high production of black pepper have been developed. Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations in black pepper. The economic optimum in terms of profitable response for money invested was found to be Rs. 1.60/standard for N, Rs. 2.40/standard for P and Rs. 5.40/standard for K. Major pests, pathogens, viruses and their insect vectors and nematodes affecting pepper were characterized and documented. Morphological and molecular characterization of black pepper isolates of *Phytophthora* further revealed that isolates shared the characters of both *P. capsici* and *P. tropicalis*.

A RNA virus, Cucumber mosaic virus (CMV) and a DNA virus, Piper yellow mottle virus (PYMoV) ware found to be associated with stunted disease of black pepper. A method for simultaneous isolation of RNA and DNA from infected black pepper plants and multiplex PCR for simultaneous detection of CMV and PYMoV in a single reaction was standardized. SYBR green based real-time PCR was developed for detection of PYMoV and CMV in black pepper. Phytoplasma with phyllody symptoms was most closely related to members of aster yellows group (16Sr I) of Phytoplasma. Integrated strategies involving cultural methods, biocontrol agents, plant products and resistant varieties were developed for the management of pests and diseases including nematodes that resulted in substantial increase in yields and pesticide free produce. Large scale multiplication of biocontrol agents such as *Trichoderma* and *Pseudomonas* for distribution to farmers for management of disease was also undertaken. The open pollinated progeny of IISR Shakthi 04-P24-1 continued to be resistant to root infection by *P. capsici* even after 4 years after planting in the field. These organisms were deposited in the national repository of microorganisms at IMTECH, Chandigarh for future reference. Species-specific primers were developed for detection of *R. similis* in soil and plant samples. The presence of β-1, 4 endoglucanase, a major secretory cellulose enzyme in nematodes, was located in *R. similis* through EST analysis. Black pepper accessions, HP-39 and Acc. 1090 were found to be resistant to nematodes besides being rich in caryophyllene. Endophytic bacteria effective against *P. capsici* and *R. similis* in black pepper have been isolated. Culture filtrates of BRB13 at 40µL mL^{-1} caused 100% mortality of *R. similis* within 24h. Basal application of *T. harzianum* and aerial spray with 1% Bordeaux mixture was found effective in controlling anthracnose disease. An integrated pest management schedule for management of root mealy bug has been developed. Metalaxyl-Mancozeb sensitivity of 81 *Phytophthora* isolates was tested and the EC50 and EC90 values ranged from 0.0002 to 14.4ppm and 1.1-68.5ppm, respectively. Among the new chemicals tested *in vitro* against *P. capsici*, Acrobat 50 showed 100% inhibition at 50ppm concentration. Profiling and activity prediction of biochemical compounds using *in silico* tools were completed for *Pseudomonas putida* BP25 and *Bacillus megaterium* BP17. PCR based techniques were developed for identification of traded black pepper and to detect adulterants in commercial black pepper powder. The existence of fungicide sensitive or resistant isolates among the field populations of *C. gloeosporioides* infecting black pepper was noticed in Pollibetta and the isolate from this locality was tolerant to recommended doses of Bordeaux mixture and carbendazim. Post harvest technologies for drying, processing, storage and production of value-added product like white pepper production were standardized.

Genomic DNA was isolated from 126 black pepper *Phytophthora* isolates and SSR profiling was done. Genetic diversity of *Phytophthora* isolates from black pepper was studied by ITS

sequencing with the universal primers ITS6 and ITS4. A native isolate of *P. capsici* (No. 98-93) infecting black pepper was completely sequenced using next generation sequencing platform, Illumina - Solexa GA II. A new database, *Phytophthora* Genome Database (http://220.227.138.212/genomedb/) based on *Phytophthora* whole genome sequencing and annotation was developed. PhytoWeb, a comprehensive portal on *Phytophthora* diseases of horticultural crops in India was developed. Phytolib, an electronic database of research publications on *Phytophthora* and database on *Radopholus* genus RADOBASE were developed and launched.

Impact studies on adoption of IISR varieties of black pepper in farmers' fields were conducted. Karshika Sankethika Darshanam and Media Meet were organized to mobilize mass media support for sharing Agro-Information. Video films on Augmenting Black Pepper Production – A Success Story (Malayalam, English, Hindi) and Success Story of a 'Prathiba' grower – Post production stage were produced.

Cardamom: IC numbers have been obtained for all the available germplasm (592 accessions) and germplasm bearing unique characters have been registered with NBPGR, New Delhi. Improved varieties such as IISR Vijetha, IISR Avinash and Appangala-1 have been developed. Two of them having mosaic or rhizome rot resistance have been popularized among the farming community. About 10 high yielding F_1 hybrids were promoted to coordinated varietal trials. Characterization of export grade cardamom from India, Sri Lanka and Guatemala based on physical, biochemical parameters and molecular techniques revealed the superiority of Indian produce for the physical parameters such as seed to husk ratio, weight of 100 capsules, number of capsules in 100g, bulk density and moisture content. GC-MS study confirmed superiority of Indian cardamom over Guatemalan and Sri Lankan cardamom. Molecular profiling of Indian cardamom revealed the existence of two genetically distinct clusters such as "Kerala cluster" and "Karnataka cluster' among the germplasm collections. High production technology has been standardized. Drip irrigation and sprinkler irrigation once in 12 days significantly improved yield attributing characters. Soil and water conservation measures have been standardized in cardamom based cropping system. Cardamom accessions APG 257, APG 414 and APG 434 were found to be promising for drought tolerance. Molecular profiles were developed for 100 accessions of small cardamom germplasm using 25 ISSR markers for studying the genetic diversity.

A procedure for total RNA isolation from cardamom and detection of CdMV through reverse transcription–polymerase chain reaction (RT-PCR) using primers designed for the conserved region of coat protein was standardized. A protocol for SYBR green based real-time RT-PCR for detection of Cardamom mosaic virus (CdMV) and Banana bract mosaic virus (BBrMV) in cardamom was developed. Surveys conducted in major cardamom growing areas of Karnataka and Kerala, revealed the prevalence of Banana bract mosaic virus (BBrMV) infection. A reliable RT-PCR based method was also developed for detection of the virus in plants. The survival of *C. gloeosporioides* infecting cardamom in infected plant part (leaves) was studied under laboratory, greenhouse and field conditions.

Ginger: Germplasm repository at IISR has the largest collection with several exotic and high quality accessions. About 668 accessions are being maintained in field germplasm conservatory. These accessions have been regularly utilized in the genetic improvement programme. An *in vitro* gene bank was established for conservation of germplasm. Three varieties namely, IISR Varada, IISR Rejatha and IISR Mahima were released for high yield and quality. Two mutants, irradiated with gamma rays showed resistant reaction even after 3 repeated inoculations with *R. solanacearum*. Ginger oil components have been character-





ized by GC-MS. A relationship between leaf P/Zn ratio and soil P/Zn ratio to rhizome yield has been established. Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations. The economic optimum in terms of profitable response for money invested was found to be Rs. 3.75/ bed for N, Rs. 1.30/ bed for P and Rs. 0.60/bed for K. Metabolite partitioning studies in three different varieties was carried out.

Post harvest technologies for processing and technologies for preparation of value added products such as salted ginger were standardized. Comparison of essential oil constituents of fresh and dry rhizomes indicated that fresh rhizomes contained higher level of monoterpenes namely, Z-citral and E-citral whereas the dry rhizomes were predominated by the sesquiterpene hydrocarbons namely, zingiberene, farnesene and sesquiphellandrene. Bacterial wilt pathogen, *Ralstonia solanacearum* in North Eastern states, Sikkim and Kerala were found similar in a molecular fingerprinting indicating strain migration from one place to another. Ginger strain of *R. solanacearum* was found to infect turmeric, cardamom, *C. aromatica, C. zedoaria, Kaempferia galanga, Zingiber zerumbet* and tomato. Indian mango ginger, *C. amada* was found to be free from bacterial wilt even under inoculated conditions. The species of *Pythium* causing rhizome rot in Kerala, Karnataka, Uttar Pradesh and Sikkim was identified as *P. myriotylum*. Nine actinomycete isolates from ginger soil were found to be antagonistic to *R. solanacearum*. Technique for seed rhizomes treatment (for elimination of bacterial wilt pathogen) and integrated disease management strategy for soft rot and bacterial wilt diseases and shoot borer was developed. *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB68) were effective for disease control and plant growth promotion. PGPR formulation to enhance nutrient mobilization and growth, yield and provide protection against diseases was developed and commercialized. The life cycle of shoot borer (*Conogethes punctiferalis*) was studied on 6 resistant and 6 susceptible accessions. The infectivity of EPNs strains IISR-EPN 01 to 08 was tested against shoot borer larvae under *in vitro* conditions. One species of EPN collected earlier from ginger soil belonged to the *Oscheius gingeri* and was identified as new species on the basis of morphological and molecular characterization. The improved varieties and technologies developed on cropping system, nutrient and water requirement, pest and disease management and post harvest processing techniques were disseminated to farmers and other agencies through publication, training programmes and demonstrations. Large scale multiplication and distribution of elite planting material were also undertaken.

Turmeric: The germplasm collected over the years have been conserved in the field gene bank and they were characterized for yield, quality, and resistance to pests, diseases and drought. Open pollinated seedling progenies generated over the years are being evaluated for their yield and quality characters. Molecular genetic fingerprints of sixteen *Curcuma sp.* using RAPD and ISSR technique revealed high degree of polymorphism among the accessions. A total of 140 microsatellites containing genomic DNA fragments were isolated adopting the selective hybridization method with di and trinucleotide biotinylated probes. Two synonymous *Curcuma* species *viz.*, *C. zedoria* and *C. malabarica* showed identical SSR profiles for 40 microsatellite loci. Seven high curcumin and high yielding varieties, Suvarna, Sudarsana, Suguna, IISR Prabha, IISR Prathibha, IISR Alleppey Supreme and IISR Kedaram were released for commercial cultivation. Efficient protocol for plant regeneration through organogenesis and somatic embryogenesis was standardized. Variations in rhizome morphology were observed among calli-regenerated somaclones indicating somaclonal variation. Accessions with high curcumin and root knot nematode resistance were identified. About forty seedling progenies with higher curcumin (>

3%) and dry recovery (> 20%) were identified. The natural enemies of shoot borer (*C. punctiferalis*) infesting turmeric were documented. Three different curcuminoids (curcumin, de methoxy curcumin and bis de methoxy curcumin) could be separated from oleoresin of rhizomes by employing chromatographic techniques. Turmeric oil components have been characterized by GC-MS. A PCR based method was developed to detect adulteration of turmeric powder with wild *Curcuma* species.

Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations. The economic optimum in terms of profitable response for money invested was found to be Rs. 0.65/ bed for N, Rs. 0.40/ bed for P and Rs. 0.85/bed for K. Increase in curcumin content was recorded when sprayed with micro nutrients like Zn and B. Processing with or without boiling or different drying methods did not lead to variation in oil, oleoresin and curcumin contents. The optimum spacing, nutrient and water requirement were standardized for different soils. Organic farming system was developed for turmeric. Basic data on distribution, bioecology, population dynamics of shoot borer (*C. punctiferalis*) and its natural enemies and crop loss due to shoot borer was generated. Lamda cyhalothrin 0.0125% was more promising in reducing the percentage of shoots infested by the shoot borer. The improved varieties and technologies were disseminated to farmers and other agencies through publications and demonstrations. The adoption of released varieties like Prathiba in AP, Karnataka and TN were studied. A novel soil pH based micronutrient mixtures for enhancing growth, yield and quality of turmeric, ginger, black pepper and cardamom were developed.

Tree spices: The germplasm holdings of 3 important tree spices, nutmeg, clove, cinnamon including cassia, garcinia and allspice are being conserved. IC Numbers for cinnamon, clove, nutmeg and allspice accessions were obtained from NBPGR, New Delhi. Cassia C1 (IC 370415) has been registered as INGR 05029 with NBPGR, New Delhi for its high oleoresin content (10.5%) besides a dwarf clove accession. The cassia elite line A1 (IC 370400) has been registered with NBPGR for high cinnamaldehyde content in bark oil (81.5%) and leaf oil (80.5%). Two high quality cinnamon varieties, IISR Navashree and IISR Nithyashree and a nutmeg variety, IISR Viswashree were released. Nutmeg accession, A11/25 was found to be promising for high yield. Nutmeg accession A9-71 (IC-537220) as a source of high sabinene (45.0% sabinene in nutmeg oil and 41.9% sabinene in mace oil) was registered with NBPGR. Tissue culture protocols have been developed for nutmeg. Protocols for DNA isolation from nutmeg have been standardized. Performance of nutmeg on *M. malabarica* continued to be better than other rootstocks for productivity. Green chip budding with orthotropic buds was standardized in nutmeg on *Myristica fragrans* rootstock with 90-100% success. GC-MS study revealed the presence of two chemotypes in *Cinnamomum verum*. Drying and processing methods for cinnamon, nutmeg and mace have been developed. Antioxidant properties and food color value are being studied in tree spices. GC-MS analysis of the chemical constituents of essential oils in leaves of *Cinnamomum sulphuratum, C. glaucescens, C. glanduliferum, C. macrocarpum* and *C. perrottetti* revealed that the major chemical constituents in these oils were α-phellandrene, β-phellandrene, camphor, t-caryophyllene and germacrene-D respectively. Vegetative propagation techniques were standardized for nutmeg, cassia and cinnamon. Major pests and diseases on tree spices were documented. The improved varieties and technologies developed on propagation and post harvest processing were disseminated to farming community.





Vanilla: The germplasm includes a flower colour variant collected from Andaman and Nicobar islands. Comparative anatomical analysis of different vanilla species was carried out. Interspecific

hybridization was made between *Vanilla planifolia* and *V. aphylla*. Reciprocal crosses were conducted between *V. planifolia* and *V. tahitensis* (species reported as resistant to root rot disease) and high percent of fruit set was observed in both the crosses. Fifty interspecific hybrids each of *V. planifolia x V. tahitensis, V. tahitensis x V. planifolia* and selfed progenies of *V. tahitensis* were established *ex vitro*. Over 1000 seed progenies of *V. planifolia* are being field tested for yield and disease resistance. Chromosome number analysis of two interspecific hybrids between *V. planifolia* and *V. tahitensis* showed 2n=30 in one (PT-5) and 2n=32 in other (PT-17).

Protocols for micro propagation through direct shoot multiplication as well as callus regeneration were standardized. Root rot and wilting were found to be the major problems in most of the plantations. Mosaic and necrosis were also observed in all the plantations and the incidence ranged from 2-80%. Cucumber mosaic virus (CMV) of vanilla was characterized on the basis of biological and coat protein (CP) nucleotide sequence properties, which showed that CMV infecting vanilla belongs to subgroup IB. A virus causing mild chlorotic mottle and streaks on leaves of vanilla was identified as a strain of Cymbidium mosaic virus (CymMV) based on coat protein gene sequence comparison and phylogenetic studies. Another virus associated with necrosis and mosaic on vanilla was identified as a strain of Bean common mosaic virus (BCMV) based on coat protein gene sequence comparison and phylogenetic studies.

Paprika: The germplasm collected from various places of cultivation were characterized for various morphological, yield and quality characters such as oleoresin, pungency and colour value. Considerable variability was observed in total extractable colour and capsaicin content (pungency) of selected paprika accessions. The lines ICBD-10, Kt-pl-19 and EC-18 were found promising with high colour value and low pungency. PCR based technique was developed to detect adulterants in commercial chilli powder.

1. BLACK PEPPER





Crop Improvement

Collection and conservation

Cultivars were collected from famer's plots of three states *viz.*, Kerala, Karnataka and Tamil Nadu. A total of 251 collections were made including 248 cultivars and 3 wild *Piper* species. A drought tolerant type was collected from Malappuram district (Figs 1.1). Spike proliferating cultivar *(Thekkan)*, other farmer bred cultivars such as *Kumbackal* and *Ponmani* are some of the unique accessions collected from Idukki district of Kerala (Figs 1.2 a & b). Acc. 7398, a cultivated black pepper having spike length up to 27.3 cm (Fig 1.3), but poor setting was collected from Yemma Gundi Estate, Suntikoppa, Madikeri. This accession has the longest spike length (average spike length 26.5 cm) among the accessions collected so far. The collections (Table 1.1) have been planted at IISR, Kozhikode under protected condition. The present status of germplasm conserved is 2936 accessions. (Wild pepper-1418, Cultivars-1509, Exotic species-9). IC numbers were obtained for 191 cultivars and 169 wild accessions from NBPGR, New Delhi. One ha area at CHES, Chettalli was planted with erythrina standards (5m x 5m spacing) for establishing a new germplasm block and planted with 426 accessions. One hundred and ten wild germplasm are being maintained at CRC, Appangala.

For the production of virus free plants of black pepper, organised shoot apex (0.3-0.5cm) comprising the apical dome and a limited numbers of leaf primordia were excised from the field grown, *in vitro* derived shoots of the variety Sreekara. The direct shoot development from meristem was achieved by regulated culture conditions so as to get the confined outgrowth of only organized shoots without any adventitious propagation. The developed shoots are now under sub culture for organogenesis to achieve full grown plantlets (Fig 1.4).

Fig 1.1 A drought tolerant accession collected from Malappuram, Kerala

Fig 1.2a *Ponmani*

Fig 1.2b *Kumbakkal*

Fig 1.3 Acc. 7398 with very long spike

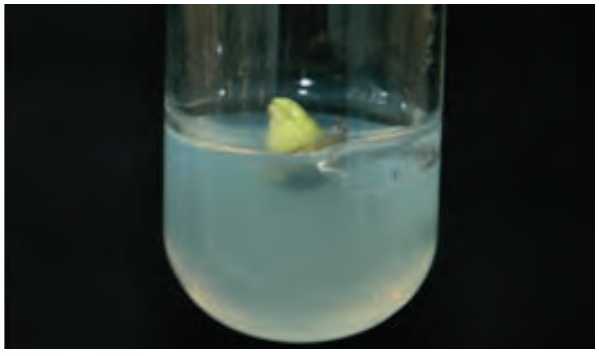


Fig 1.4 Well grown meristems as shoot tips after sub culturing in MS+BA+NAA (10 day old cultures)

Table 1.1 Diversity of black pepper accessions collected

S. No	Name of the cultivar	No. of accessions collected	S. No	Name of the cultivar	No. of accessions collected
1	*Arakulamunda*	13	21	*Malamundi*	1
2	*Arayan mundi*	4	22	*Marampadathi*	1
3	*Arivally*	3	23	*Multibranched*	1
4	*Arka Coorg Excel*	1	24	*Mundi*	3
5	*Balankotta*	23	25	*Narayaakodi*	2
6	*Cheriyakaniakadan*	2	26	*Neelamundi*	12
7	*Cherukodi*	2	27	*Orumaniyan*	1
8	*Cholamundi*	1	28	*P-1*	2
9	*Chumala*	2	29	*Perumkarimunda*	1
10	*Irumanian*	3	30	*Perumkodi*	2
11	*Jeerakamundi*	8	31	*Ponmani*	3
12	*Kalluvally*	11	32	*Poonjaranmunda*	4

13	*Kaniakadan*	12	33	*Thevanmundi*	6
14	*Karimunda*	22	34	*Thommankodi*	1
15	*Karuvilanchi*	1	35	*Vadakkan*	3
16	*Kottanadan*	5	36	*Valiyakaniakkadan*	2
17	*Kumbackal*	2	37	*Vattamundi*	2
18	*Kurialmundi*	1	38	*Vellamundi*	2
19	*Kuthiravally*	2	39	*Vellanamban*	1
20	*Local types*	78	40	*Wayanadan*	2
Total			248		

Interstock grafting

Since *Piper* accession (Acc. 5815) resistant to *Phytophthora* and nematodes is incompatible with black pepper, *P. colubrinum* and *P. hamiltoni* were tried as interstock for variety Sreekara because these 2 species are not only disease resistant but also compatible with pepper. The survival and growth of Sreekara on these combinations were very poor and hence found not promising. Though *P. longum, P. hymenophyllum, P. attenuatum, P. sermantosum, P. hapnium, P. argyrophyllum* and *P. chaba* were initially successful on Acc. 5815 these subsequently failed. However, the grafts of *P. ornatum* with *P. chaba* had shown excellent growth and fruiting even after 12 months indicating good success. So *P. ornatum*, a resistant species may be used as rootstock for *P. chaba* where foot rot is a problem. Grafts of Sreekara on *P. hamiltoni* died during summer under rainfed conditions. Hence, similar to *P. colubrinum P. hamiltoni* as rootstock is feasible only under irrigated conditions limiting its scope. The resistant species *P. ornatum* and Acc. 5815 cannot be utilized directly for black pepper through grafting but may be useful as material for resistance breeding.

The hybrids (pollu beetle resistant) developed from various cross combinations were multiplied and maintained in the nursery. The hybrids multiplied are (1) Acc. 816 x Subhakara-80 (2) Acc. 1084 x Subhakara-50 (3) Acc. 841 x Subhakara-30 and (4) Acc. 1114 x Subhakara-30.

DNA barcoding to discriminate adulterants

Standardized 8 barcoding loci (mat K, rbcL, trnH-psbA, ITS, atpH-atpF, psbK-psbI, rpob, rpoC1) for *Curcuma* species (*C. longa, C. xanthorrizha, C. zedoria*), *Piper* sp. (*P. nigrum, P. atteunatum, P. galeatum*), chilli (*Capsicum annum*) and *Cinnamon* species (*C. verum, C. cassia, C. malabathrum*) and 4 barcoding loci (mat K, rbcL, trnH-psbA, ITS) were selected based on their discriminatory power.

In the black pepper group comprising 5 accessions, one each of *P. nigrum, P. atteunatum, P. galeatum*, 4 different varieties of papaya and 5 branded market samples, the selected 4 barcoding loci were amplified and the products were gel purified and sequenced bidirectionally. The sequences were aligned using Clustal W, trimmed by Bioedit software and analysed using MEGA 5. Single Nucleotide Polymorphisms (SNPs) were found to be higher in trnH-psbA locus and was found to be the best for adulteration detection.

Detection of adulterants in market sample was done using trnH-psbA locus. Out of 5 branded pepper market samples, an adulterant specific band (~600bp) could be observed in one of the samples. Sequence analysis (NCBI Blast) revealed that this band has high similarity with capsicum (Fig 1.5). Further validation of the locus was done by checking with simulated samples of chilli: pepper in the ratio 1:10, 1:25, 1: 50, 1:100, 1:200. In





all the simulated samples the adulterant specific band (~600bp) was amplified (Fig 1.6).

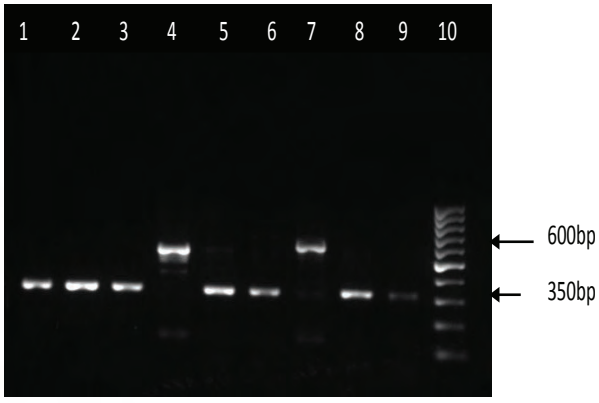


Fig 1.5 Amplification of trnH-psbA locus(Lane 1- *Piper nigrum*, lane 2- *Piper attenuatum*, lane 3- *Piper galeatum*, lane 4- papaya, lane 5 to 9-Market samples, lane 10-100bp ladder)

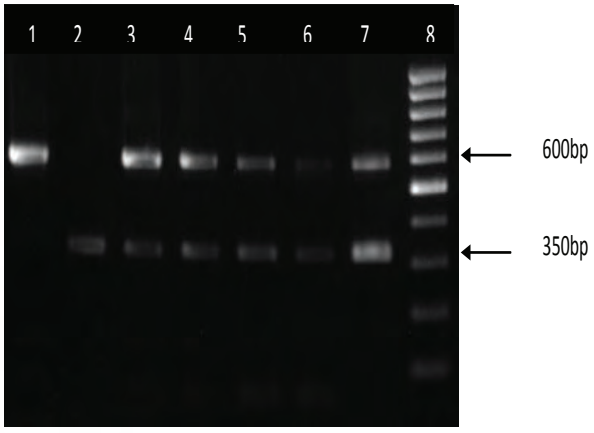


Fig 1.6 Amplification of adulterant specific bands in simulated samples and controls (Lane 1- *Capsicum annum* (positive control), lane 2- *Piper nigrum* (negative control), lane 3-simulated sample (1:10), lane 4- simulated sample (1:25), lane 5- simulated sample (1:50), lane 6- simulated sample (1:100), lane 7- simulated sample (1: 200), lane 8 - 100bp ladder.)

Expression profiling of genes induced under water stress

Expression analysis of candidate genes for drought tolerance by qPCR was undertaken in Acc. 4226, tolerant to water deficit conditions by comparing the expression under water deficit and controlled conditions. Among the genes studied, DREB like proteins potentially associated with stress-responsive transcriptional regulation was found to be highly expressed under water deficit. Slight increase in expression of a specific BADH, thought to be involved in osomoprotectant metabolism and the molecular chaperone, HSP 70 was also found (Fig 1.7). However, the expression of a specific MAPK was found to be reduced under stress and bzip did not show any significant change.

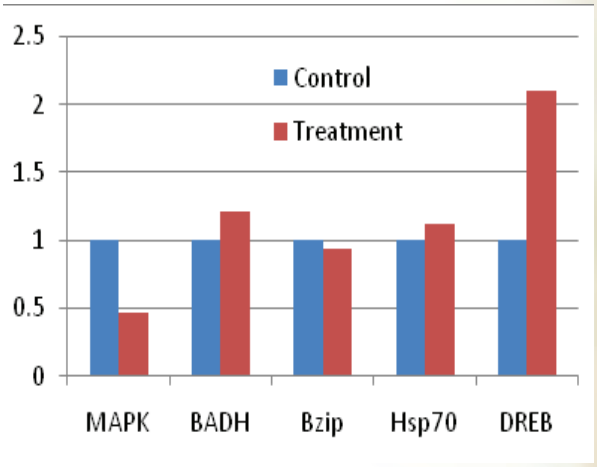


Fig 1.7 Relative expression of certain drought responsive genes, MAP kinase, BADH- (Betaine Aldehyde dehydrogenease), bZIP transcription factor, Hsp70 and DREB (Dehydration responsive element binding protien) under water deficit stress

Host pathogen interaction

The transcriptome data analysis derived from *P. nigrum* and *P. colubrinum* leaves challenged with *P. capsici* revealed identification of many defense related genes differentially expressed in these 2 different species of *Piper*.

Sequences with homology to Lectin-like receptor kinase a potential host target for RxLR type of effectors from *Phytophthora* was discovered and over expression of this gene was found to be useful in other crops for enhanced resistance to *Phytophthora*. Other components for induced defense system, the small peptides with antimicrobial activity such as defensins were also discovered. Sequences with homology to Polygalacturonase inhibitor protein (PGIP), a defense

gene, potential to be used in the resistance breeding were identified. Defense-associated transcription factors belonging to the family of NAC, BHLH, MYB, MYC, BZIP etc. that can act downstream reactions of defense-related genes were also recovered.

Analysis of allelic variants in selected genes (Table 1.2) revealed maximum SNPs in MAP kinase gene compared to other genes like PAL, Polyphenol oxidase, etc.

Table 1.2 SNPs discovered in specific genes involved in *Piper colubrinum – Phytophthora* interaction

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

Molecular analyses of the expression of five defense-associated genes were carried out using real-time quantitative reverse transcription-polymerase chain reaction (QRT-PCR) assays. The expression profiles of different defense related genes differed significantly in the studies undertaken in *P. colubrinum* leaves challenged with *P. capsici.* The fold expression was very high in case of Polygalacturonase inhibitor protein, followed by Polyphenol oxidase, Thaumatin-like protein, defensin and Cysteine proteinase inhibitor gene (Fig 1.8).

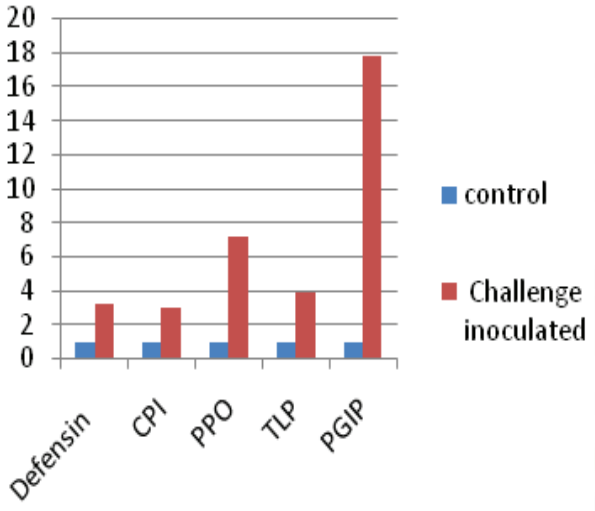


Fig 1.8 Relative expression of defense related gene in *Piper colubrinum* challenge inoculated with *Phytophthora* (CPI-Cysteine proteinase inhibitor, PPO-Polyphenol oxidase, TLP-Thaumatin-like protein and PGIP-Polygalacturonase inhibitor protein)





Host resistance

ISSR profiling of progenies of P1 × Subhakara crosses

Twenty ISSR primers have been screened with parents-Panniyur1 and Subhakara. 10 Primers that showed polymorphism between parents was used to profile 94 selected progenies of Panniyur1 × Subhakara (Fig 1.9 & 1.10).

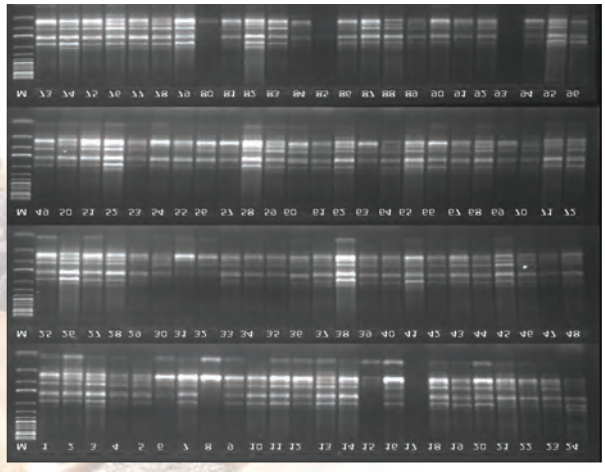


Fig 1.9 Agarose gel showing amplification of mapping population using UBC834a–(AG)8CT with 1Kb Marker ladder

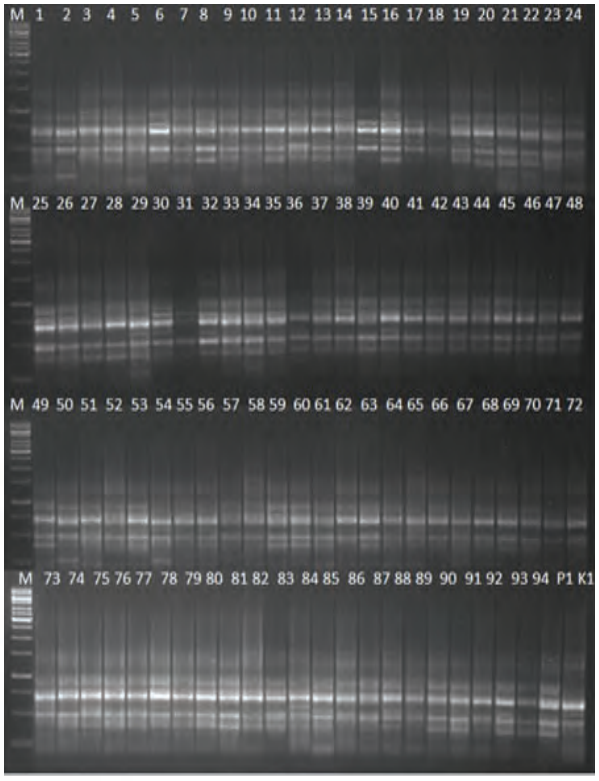


Fig 1.10 Agarose gel showing amplification of mapping population using C1:-CAGC(AC)7 with 1Kb Marker ladder

Amplification of resistance gene candidates

The primer pair, P6F- 5'-GGACCTG-GTGGGGTTGGGAAGACAAC-3' and P6 R-5'-CAACGCTAGTGGCAATCC-3' was used to amplify R gene candidates. The selected set of primers were designed according to the conserved P-loop (GGVGKTT) and hydrophobic domain (GLPLAL) from the N, L6 and RPS2 genes of the NBS-LRR class specific against pathogens (Fig 1.11).

The wild species *P. colubrinum* (Acc. 392) and *P. ornatum* (Acc. 3362) and the moderately resistant varieties IISR Sakthi and P24-O-4 and susceptible varieties, Subhakara and Sreekara were used in this study.

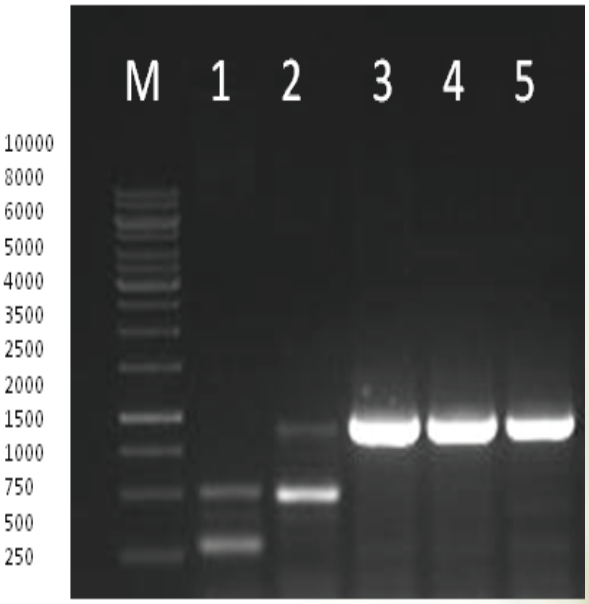


Fig 1.11 Agarose gel (1.5%) showing amplified products of primer combinations, P6 F and R:-M-1Kb, 1) *P. colubrinum*, 2) IISR Sakthi, 3) P24-O-4, 4) Sreekara, 5) Subhakara.

Sequence analysis of *Piper* RGAs

Moderately tolerant varieties (IISR Sakthi, P24-O-4) and susceptible varieties (Subhakara and Sreekara) were amplified using degenerate primers designed from known R genes (N, RPS2). The presence of conserved domains, *viz.*, kinase-1a, kinase-2 and hydrophobic motif provided evidence that the sequences belong to the NBS-LRR class gene family. The presence of tryptophan as the last residue of kinase-2 motif further qualified them to be in the non-TIR NBS-LRR subfamily of resistance genes. *P. nigrum* RGAs were compared with *P. colubrinum* RGA and *P. nigrum* NBS analogs formed 2 groups with *P. colubrinum* resistance gene analogs (Table 1.3 and Fig 1.12).

Piper RGA sequences derived using the R-gene specific degenerate primers have been deposited in the GenBank database under the accession numbers: JX416288-JX416291 and JX898031 - JX898033.

Table 1.3 Similarity between *P. nigrum* and *P. colubrinum* NBS sequences and other GenBank accessions

Piper RGA	Conserved domain	Amino acid identity
P. colubrinum	NB-ARC	resistance protein-like [*Vitis bashanica*] 48% ,2e-11
P. nigrum (IISR Sakthi)	NB-ARC	resistance protein-like protein, partial [*Citrus trifoliata*], 42%,2e-34
P. nigrum (P24-O-4)	NB-ARC	NBS-LRR class resistance protein [*Solanum trilobatum*], 49%,2e-46
P. nigrum (Sreekara)	NB-ARC	NBS-LRR class resistance protein [*Solanum trilobatum*] 49%, 8e-47
P. nigrum (Subhakara)	P-loop NTPase	NBS-LRR disease resistance-like protein [(*Populus tomentosa* x *P. bolleana*) x *P. tomentosa* var. truncata] 46%,8e-42

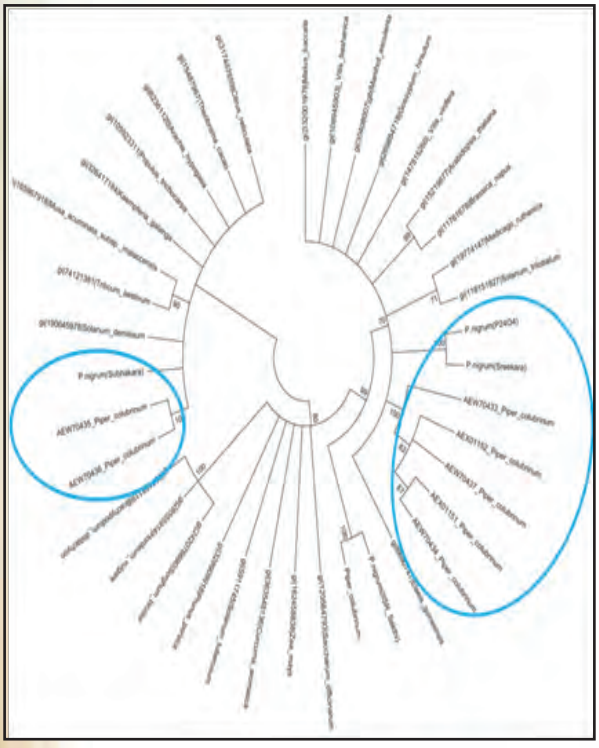


Fig 1.12 Average distance tree using % identity of amino acid sequences showing relationship of *P. nigrum* and *P. colubrinum* NBS analogs with the characterized NBS domain of R-genes from other plant species

Crop Production

Tree species-black pepper interaction

In the green house study on the allelopathic effect of tree standards on growth of bush pepper, the leaf and stem extracts of tree species like garuga, erythrina, gliricidia, ailanthus, jack and silver oak at 100% concentration was applied to bush pepper @100mL per plant twice every month.

Observations on growth parameters taken after eight months of application revealed little or no significant differences among the treatments including control. The data on soil parameters revealed that soil pH was near neutral in all treatments. Available nutrient levels including organic C were markedly high in all the treatments. Dehydrogenase level was lowest in the control and silver oak, but markedly higher in soils applied with other tree extracts. Microbial biomass C was lowest with silver oak and ailanthus extracts but greatest with garuga extract (Table 1.4).

Table 1.4 Effect of tree sp on pH, available P & K, organic C, microbial biomass C and dehydrogenase activity in soils under bush pepper

	pH (1: 2.5 H_2O)	Bray P (mg kg^{-1})	Exch. K (mg kg^{-1})	Organic C (%)	Microbial biomass C (mg kg^{-1})	Dehydrogenase (µmol TPF g $soil^{-1}$ h^{-1})
Ailanthus	7.04	234	810	5.53	610	0.89
Garuga	7.11	307	809	5.76	2071	0.70
Gliricidia	7.10	244	428	5.43	1020	0.61
Erythrina	7.03	252	544	5.63	1084	0.63
Jack	7.05	272	660	6.00	1264	0.60
S. oak	6.99	275	508	5.16	285	0.37
Control	7.07	337	867	5.83	1028	0.42
lsd (0.05)	NS	65	165	0.72	1670	0.37









Microbial consortium for growth promotion

A consortium of three rhizobacterial isolates (BRB3, BRB13 and BRB23) was found to markedly promote the growth of black pepper. However, the PGPR applied in combination with various rates of chemical fertilizers differed in their effects on black pepper growth. The soil microbial activity measured through various parameters was consistently greater in treatments involving PGPR. However, the PGPR treatments also varied in their effects on soil microbial properties. Microbial biomass N and acid phosphatase activity were greatest in the treatment 100%N + 75%P + 100%K + BRB3 + BRB13, microbial biomass C in the treatment 100%N + 100%P + 75%K + BRB3 + BRB23, microbial biomass P in the treatment 75%N + 100%P + 100%K + BRB13 + BRB23 and β glucosidase in the treatment 100%N + 100%P + 100%K + BRB3 + BRB23. Based on a series of experiments, a microbial formulation involving this consortium of PGPR was developed for licensing and commercialization.

Screening germplasm accessions for drought tolerance

One hundred black pepper germplasm accessions were screened for drought tolerance based on relative water content (RWC) and membrane leakage (ML). Among the accessions, accessions 6707 and 6720 maintained >70 % RWC and <9 % ML after 15 days of stress induction. Soil moisture after 15 days of stress was 8-8.4%.

Acc No.	DAS	RWC (%)	ML (%)	Soil moisture (%)
6707	Control	94.7	4.9	20.5
	6 DAS	87.1	7.3	15.6
	15 DAS	84.1	8.7	8.4
6720	Control	91.5	5.5	21.0
	6 DAS	84.4	7.9	15.2
	15 DAS	79.8	8.5	8.0

Field testing of germplasm accessions for drought tolerance

Ten previously identified drought tolerant accessions (acc nos. 1439, 1622, 807, 4072, 1277, 971, 4226, 1495, 1368 and 931) along with Subhakara (check) have been planted in the field at Chelavoor farm for further evaluation.

Organic farming

An organic package involving FYM, vermicompost, ash and rock phosphate combination and *Azospirillum* and *Phosphobacteria* and *Trichoderma* sp. and *Pseudomonas* sp. was developed and recommended. For black pepper, an organic package consisting of application of 10 kg FYM + 500g neem cake + 500 g ash+ 2 kg vermicompost with biofertilizers – *Azospirillum* and P solubilizing bacteria (20g) and *Trichoderma* sp. (50g) and *Pseudomonas* sp. (IISR 6) (50 g) per vine and spray of BM and neem oil has been standardized and demonstrated.

Soil carbon buildup under spices based cropping systems

Soil samples were collected from two depths (0-15 and 15-30 cm) in pure cardamom, cardamom + coffee + black pepper, coffee + black pepper systems in Madikeri and Wayanad districts and also from black pepper maintained under organic, integrated and chemical management systems. The wet oxidizable C ranged from 1.3– 3.1g $100g^{-1}$ in 0-15 cm layer and 0.69-2.1g $100g^{-1}$ in 15-30 cm layer. The total organic C (TOC) content was 4.31, 3.73 and 3.41g $100g^{-1}$ in the 0-15 cm soil layer of black pepper managed under organic, integrated and inorganic systems, respectively. The total organic N (TON) content was higher under organic management (1.61, 1.35g $100g^{-1}$) followed by integrated (1.46, 1.19g $100g^{-1}$) and inorganic (1.34, 1.35g $100g^{-1}$) management systems of black pepper, at surface and sub surface layers. TOC:TON ratio was observed to be in the range of 2.20-2.75.

The particulate organic C content varied

from 0.91 – 1.83g $100g^{-1}$ in the top soil and 0.53 - 1.25g $100g^{-1}$ in the sub surface layer under different management systems. The particulate organic N was higher under organic management (0.83, 0.38g $100g^{-1}$) followed by integrated (0.74, 0.21g $100g^{-1}$ and inorganic (0.32, 0.32g $100g^{-1}$) management systems. The ratio of POC/PON varied from 2.1-3.6. The POC contributed only 23-31% of TOC, where as the wet oxidizable C fraction contributed 59-71% of TOC at different depths and management systems. The POC fraction also accounted for only 35-47% of wet oxidizable C fraction.

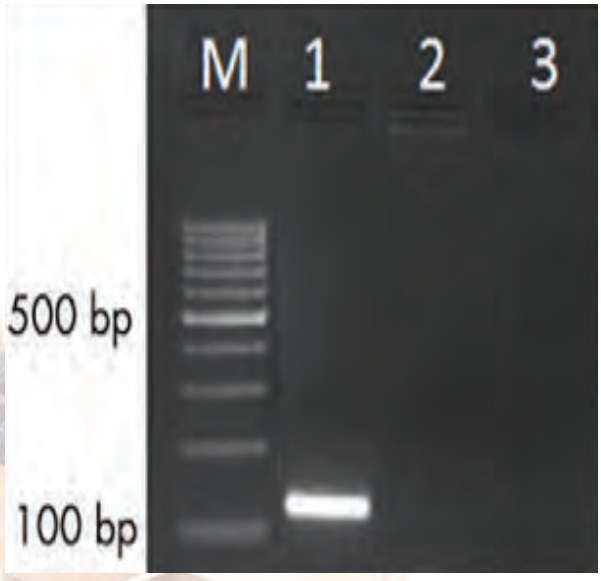
Coffee + Pepper system recorded TOC content of 4.29g $100g^{-1}$ in the 0-15 cm layer followed by Cardamom alone (3.63g $100g^{-1}$) and Coffee + Cardamom + Pepper recorded the least (2.5g $100g^{-1}$). The TON content also followed a similar pattern in the surface and sub surface soils. The wet oxidizable C fraction accounted for about 29-71% of the TOC in the systems studied.

Crop Protection

Development of Real-time PCR for viral detection

A SYBR Green based quantitative real time polymerase chain reaction (qPCR) for *Piper yellow mottle virus* (PYMoV) and reverse transcription (RT) qPCR for *Cucumber mosaic virus* (CMV) infecting black pepper was developed for sensitive and specific detection and quantification of viruses. The primers were designed to conserved regions in both the viruses (coat protein in CMV and ORF III in PYMoV) identified by Clustal x alignment. The qPCR and RT-qPCR was performed on a Rotor Gene Q real-time system (Qiagen, Hilden, Germany). The qPCR reaction for PYMoV was carried out in a final volume of 25µL each containing: 12.5µL of 2x QuantiFastTM SYBR Green PCR Master mix, 1.0µL of each forward and reverse primer (1µM µL) and 1µl template (about 60ng). Thermocycling conditions used were an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 95°C for 15s and 60°C for 45s. The components of RT-qPCR reaction for CMV consisted of 50U of Revert Aid reverse transcriptase in addition to the above components and thermocycling conditions included an additional step of cDNA synthesis at 42°C for 45 min at the beginning. Healthy black pepper total nucleic acid and water control (without template) were used as negative controls to monitor specificity of the primers and potential contamination within the real time PCR reagents. Following qPCR and RT-qPCR, the amplicons were subjected to melt analysis from 60-95°C. The specificity of the qPCR and RT-qPCRproduct was verified (apart from melt curve analysis) periodically using a 1.5% agarose gel electrophoresis.

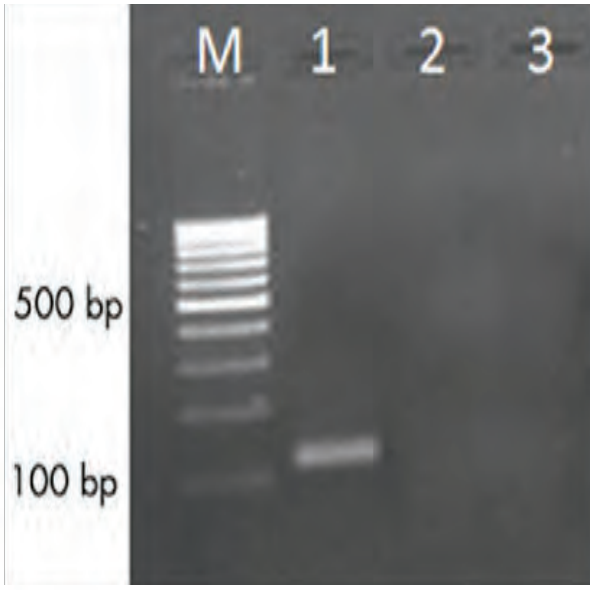
The qPCR and RT-qPCR primers amplified expected product from known infected plants that were previously analysed by PCR and RT-PCR while no amplification was seen for healthy sample and water control. The melt curve of qPCR and RT-qPCR products for infected black pepper samples had a single peak at 82.5- 83.0°C indicating that only the target fragment was amplified. The healthy and water control showed no melting peak. The specificity of the product was also confirmed by agarose gel electrophoresis which showed single band at expected size (Fig. 1.13).



(A)







(B)

Fig. 1.13 Agarose gel electrophoresis of Quantitative real time PCR (qPCR) for the detection of virus (A) Piper yellow mottle virus (B) Cucumber mosaic virus Lane 1: Infected black pepper; Lane 2: healthy black pepper; Lane 3: water control. Lane M shows 100 bp DNA ladder

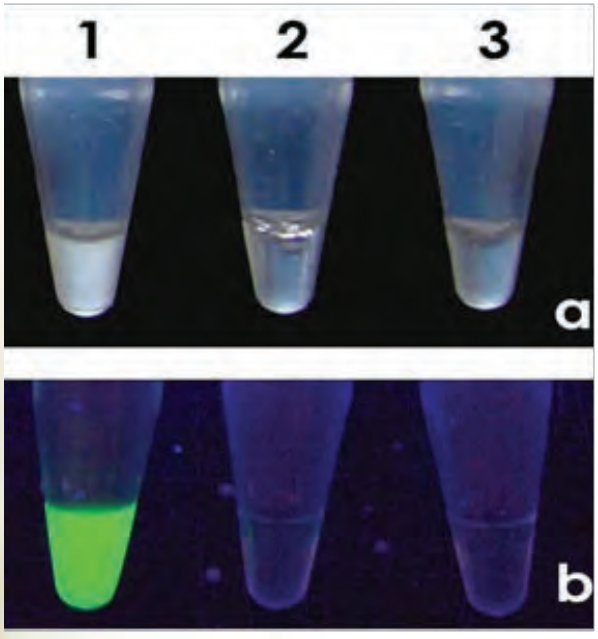
In order to develop standard curve for quantification, the target regions from both the viruses were amplified and cloned in plasmid vector. Yield of recombinant plasmid DNA was calculated using a spectrophotometer and copy number of recombinant plasmid was calculated. The recombinant plasmid was then diluted serially and subjected to qPCR as explained above. The output data were analyzed with the software available in Rotor Gene Q system (Qiagen). A standard curve for each virus was constructed by plotting Ct values from the assays with standard dilution versus the logarithm of the concentration for the exponential phase of the reaction.

The sensitivity limit and range of the SYBR Green qPCR and RT-qPCR assays were deduced from the standard curves for each virus using plasmid standards. The amplification of the standard dilution series yielded in linear relations with reliable results (R^2 = 0.99984 for CMV and R^2 = 0.99968- for PYMoV) in the range of 10 – 10^8 copies of target DNA locus per PCR. The detection limit was as low as 26 copies for CMV and 24 copies for PYMoV as estimated from the standard curve equation.

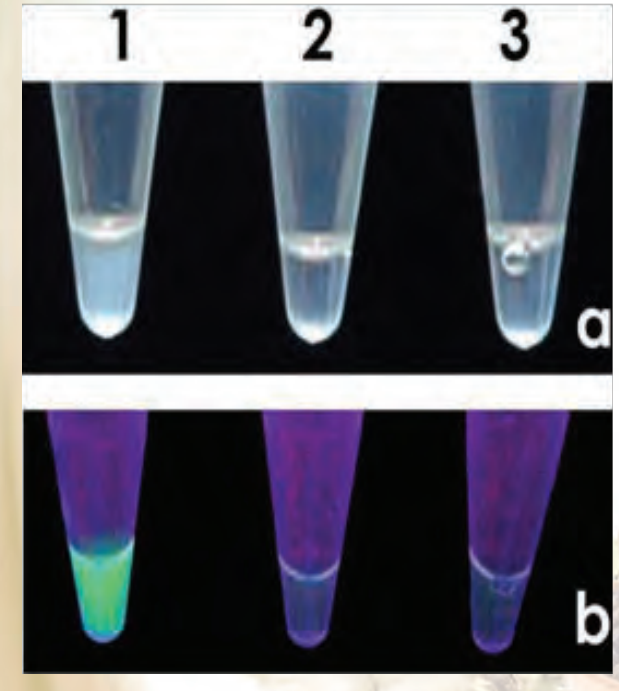
In order to know detection limit of the viruses in plants, total nucleic acid from infected plant was diluted 10-fold and was subjected to qPCR and RT-qPCR using 1 μL^{-1} of each dilution. The detection limit (copy number) of CMV and PYMoV was determined from the standard curve using Ct values obtained. The detection limit was as low as 14 and 7 copies (dilution 10^{-4} and 10^{-3}) for CMV and PYMoV respectively as estimated from the standard curve equation. The developed qPCR and RT-qPCR assays were used to detect virus in 174 symptomatic and non-symptomatic field samples of black pepper, long pepper and betel vine collected from different agro-climatic regions. All the symptomatic samples were either infected by one or both the viruses while non symptomatic plants tested were infected with a single virus.

Development of LAMP for detection of viruses

A quick detection assay based on loop mediated isothermal amplification (LAMP) was developed for detection of PYMoV and reverse transcription (RT) LAMP for detection of CMV infecting black pepper. Each LAMP and RT-LAMP assay used a set of five primers designed against conserved sequences located in the viral genome. Total nucleic acids containing both RNA and DNA isolated from black pepper was used as template for assays. The LAMP and RT-LAMP were carried out at 65°C for 60 min. The results of LAMP and RT-LAMP reaction was assessed visually by checking turbidity, pellet formation and green fluorescence in the reaction tube and also by gel electrophoresis. The assay successfully detected both the viruses in infected plants while no cross-reactions seen with healthy plants (Fig. 1.14). The optimized LAMP and RT-LAMP were validated by testing field samples collected from different regions.



(A)



(B)

Fig. 1.14 (A) Loop mediated isothermal amplification (LAMP) for the detection of *Piper* yellow mottle virus (B) reverse transcriptase (RT) LAMP for detection of cucumber mosaic virus infecting black pepper. Lane 1: Infected black pepper; Lane 2: healthy black pepper; Lane 3: water control. Visualization (a) normal light (b) UV light

Screening against *Piper* yellow mottle virus (PYMoV)

Out of 1992 germplasm accessions screened for resistance against *Piper* yellow mottle virus, four accessions showed resistance the preliminary tests.

Genome sequencing and annotation

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

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

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





Conserved domain search to identify the protein families present in exonic regions of whole genome sequences of the 2 different isolates of *Phytophthora* sp. infecting black pepper (05-06 and 98-93) has been carried out along with Blast2GO analysis.

The evolutionary history of the *Phytophthora* genomes sequenced was traced out using information from multilocus gene trees for 4 mitochondrial and 10 nuclear markers from 6 closely related species of Clade 2 *viz*., *P. tropicalis, P. capsici, P. citricola, P. citrophthora, P. botryosa, P. meadii*, along with 05-06 and 98-93 IISR isolates. Our concatenation-based multispecies coalescent approach using Bayesian, maximum parsimony and maximum likelihood methods was able to estimate a moderately supported species tree showing a close relationship among 05-06 with *P. capsici* and 98-93 with *P. tropicalis*.

In vitro evaluation of fungicides against *C. gloeosporioides*

In vitro evaluation of fungicides on the growth of *C. gloeosporioides* from necrotic lesion bits obtained from the runner shoots showed that the combination product of carbendazim and mancozeb (Fig 1.15) was more inhibitory to the growth of the fungus compared to Bordeaux mixture and carbendazim.

Fig 1.15 *In vitro* evaluation of fungicides against *C. gloeosporioides*

Management of anthracnose disease in nursery

The technology consisting of pre-planting treatment of three node cuttings prepared from the runner shoots with fungicides to manage anthracnose disease under nursery conditions was validated. The combination product of carbendazim + mancozeb was found to be superior in delaying disease initiation and development (Table 1.5).

Table 1.5 Nursery technology against anthracnose disease in nursery

Treatments	Percent Disease Index					
	6WAP	7WAP	8WAP	9WAP	10WAP	11WAP
Ridomyl	12.22	11.10	26.66	29.88	38.88	41.10
Carbendazim + Mancozeb (C+M)	6.66	18.88	31.10	22.22	45.55	51.11
(C+M) + Ridomyl	0	8.88	19.99	23.32	26.65	23.33
Propiconazole	19.99	36.66	46.66	48.88	69.99	73.33
Propiconazole + Ridomyl	17.77	36.66	49.99	54.44	58.88	61.11
Carbendazim	11.09	32.22	45.55	46.66	59.99	65.55
Carbendazim + Ridomyl	11.11	32.22	49.99	58.88	63.33	64.44
Copper oxy chloride	83.33	91.10	91.10	93.33	93.33	94.44
Copper oxy chloride+ Ridomyl	54.44	43.33	54.44	63.33	65.55	71.10
Hexaconazole	11.10	29.97	32.21	37.77	42.21	47.77
Hexaconazole + Ridomyl	18.88	44.44	57.77	66.66	74.44	79.99
Bordeaux mixture	67.77	52.33	79.99	84.44	86.66	86.66
Bordeaux mixture + Ridomyl	31.10	43.33	59.99	67.77	71.10	74.44
Untreated control	57.77	81.10	90	91.11	91.11	94.44

	SEd	CD (0.05%)	CD (0.01%)
Fungicides (F)	2.75	5.43**	7.16
Weeks (W)	1.8	3.55**	4.70
F x W	6.73	NS	17.56

Characterization of endophytic bacteria

Endophytic bacteria such as spontaneous Rifampicin resistant *Bacillus megaterium* BP-17R and *Pseudomonas putida* BP-25R were characterized by Biolog, biochemical and antibiotic sensitivity assays. Reactions of these bacteria to 17 antibiotics indicated that *P. putida* BP-25R is resistant to several antibiotics while *B. megaterium BP-17R* was resistant only to Rifampicin (Table 1.6).

Table 1.6. Reaction of *Bacillus megaterium* BP-17 R and *Pseudomonas putida* BP-25R to 17 antibiotics

Name of the antibiotic	Zone of inhibition (mm)	
	BP-17R	BP-25R
Rifampicin (30µg)	R	R
Ampicillin (30µg)	25	R
Chloramphenicol (30µg)	23	R
Polymyxin B sulphate (100u)	17	15
Amoxicillin (30µg)	24	R
Doxycyclin hydrochloride (30µg)	30	17
Trimethoprim (30µg)	23	R
Nalidixic acid (30µg)	20	R
Cephalothin (30µg)	37	R
Kanamycin (30µg)	28	20
Chlorotetrcyclin (30µg)	28	21
Ciprofloxacin (30µg)	26	29
Cephotaxime (30µg)	31	14
Cephalexin (30µg)	35	R
Oxacillin (30µg)	25	R
Novobiocin (30µg)	25	R
Lincomycin (50mcg)	11	R

Screening of bacterial metabolites

The secondary metabolites of *P. putida* and *B. megaterium* were screened for their activity against fungi, protozoa and oomycetes and for their antihelminthic activity, respectively, using in silico tools, *in vitro* and *in planta* bioassays. Eighty nine compounds from *P. putida* and 131 compounds from *B. megaterium* were predicted to possess anti-oomycete and nematicidal properties, respectively. The predicted compounds from *P. putida* were further docked with Glucanase Inhibitor Protein of *P. capsici* while the compounds of *B. megaterium* were docked with 1, 4 beta endoglucanase enzyme of *R. similis*. The most promising compounds in *P. putida* after the docking study using the above target are 2-octaprenylphenol, 2-methoxy-6-(all-trans-octaprenyl) phenol, canavaninosuccinate and 5-amino-1-(5-phospho-D-ribosyl) imidazole. In *B. megaterium*, the highest docking results were obtained with (S)-methylmalonate-semialdehyde, 2-methoxy-6-(all-trans-octaprenyl) phenol, phenyl acetate, 1-palmitoyl glycerol 3-phosphate, tributryin and 5-[(1- phenylcyclohexyl)amino] pentanoic acid. The presence of these compounds was confirmed by LC-MS analysis and genomic approaches in *P. putida*. However, whole metabolome extracted from BP-17R and BP-25R on assaying against *P. capsici, P. tropicalis, P. myriotylum, Rhizoctonia, Fusarium, R. solanacearum* and nematodes by *in vitro* bioassays did not show any activity against any of the tested pathogens, including nematodes. However, at a concentration of 20mg mL^{-1} these extracts inhibited germination of *P.capsici* zoospores (Table 1.7). Preliminary *in planta* assays against *P. capsici* using the crude metabolites were also negative.

Colonization of *P. putida* in black pepper

Colonization studies using different concentrations of gfp tagged *P. putida* (BP-25R::gfp 58) proved the endophytic nature of the bacterium. The bacterial cell density in cut shoots increased as the initial bacterial concentration was increased from 10^6-10^{10} cfu mL^{-1}, the population being lower at the distal end. However, on inoculating black pepper rooted cuttings, the bacteria colonized mostly roots, the population directly proportional to the duration of inoculation. Bacterial colonization on stem region was noticed only at the 28th day. Below detectable populations of the bacteria ware observed in leaves of treated rooted cuttings.

Extraction and purification of phenylpropanoids

Qualitative and quantitative assays were carried out for anthocyanidins, proanthocyanidins and their precursors, leucoanthocyanidins, in root extracts by specific extraction procedures and butanol/HCl assay. Levels of leucoanthocyanidins are found to be higher in the susceptible cultivar (Sreekara) while levels of condensed tannins (proanthocyanidins and anthocyanidins) and phenols were higher in the resistant line, Hp 39 (Fig. 1.16). Butanol/HCl assay was carried out in a few more selected lines reported as resistant to plant parasitic nematodes. The results have indicated that the level of proanthocyanidins was comparatively high in resistant lines *viz*., Acc. 3219, C.1090 and *P. colubrinum* compared to susceptible lines like Sreekara or Panniyur 1.









In silico and *in vitro* assays

The *in silico* results were further refined by selecting additional targets like calreticulin 1, xylanase, transthyretin-like protein 3 precursor, cathepsin B, cytochrome c oxidase subunit III and cathepsin S-like cysteine proteinase (Fig. 1.17). Syringin, sinapaldehyde, sinapic acid, scopolin and caffeoylquinic acid consistently showed good potential with high mol dock score and higher number of hydrogen bonds. Subsequently, *in vitro* bioassays were conducted with 6 new compounds *viz.*, catechol, gallic acid, phloroglucinol, salicylic acid, syringaldehyde and tannic acid. Out of the 6 compounds, syringaldehyde and salicylic acid caused >80% mortality of *R. similis* within 48h of exposure.

Localization of phenyl propanoids

Phenolic cells and lignified walls were visualized in root sections of both HP39 and Sreekara by applying histochemical staining. Phenyl propanoids were localized in roots through staining with diphenylboric acid 2 aminoethyl ester (DPBA) coupled with fluorescence microscopy. Increased presence of phenyl propanoids was observed in the cortical region of HP39 roots, consequent to nematode inoculation. Lignin was spotted in root sections through two methods *viz.*, Maule staining and Weisner staining. In general, lignification is significantly high in HP39 roots compared to Sreekara indirectly supporting the inhibition of nematodes by phenyl propanoids in the lignin pathway.

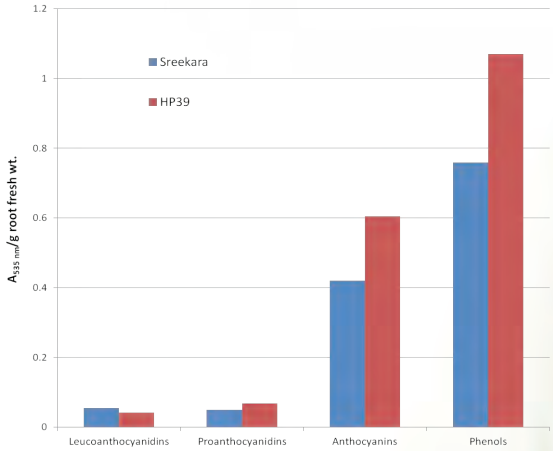


Fig 1.16 Comparative levels of phenyl propanoids in black pepper lines, Sreekara and Hp 39

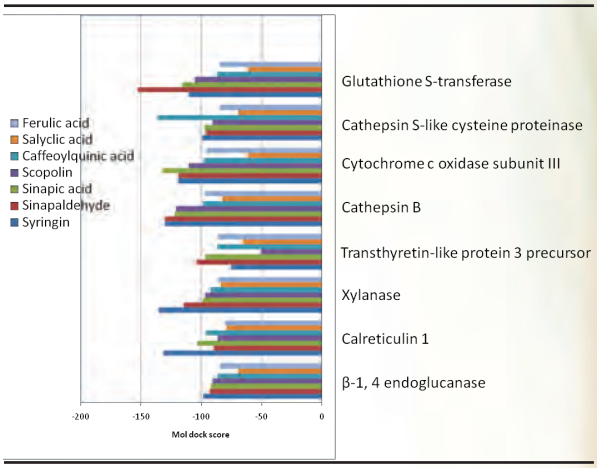


Fig 1.17 Results of *in silico* screening of a few phenyl propanoids against different targets in *Radopholus similis*

Table 1.7 Effect of bacterial metabolites on germination of zoospores of *P. capsici*

Treatments	Zoospore germination (%)				Mean germ tube length (µm)		
	2h	4h	6h	Mean	2h	4h	6h
BP-17 R Butanol	39.15 (6.257)	50.31 (7.093)	53.48 (7.313)	47.44 (6.888)	18.20*	26.20	30.70
BP-17 R Ethyl acetate	16.70 (4.087)	38.78 (6.227)	50.03 (7.073)	33.59 (5.796)	14.58	33.02	49.50
BP-25 R Butanol	21.22 (4.607)	45.47 (6.743)	52.32 (7.233)	38.37 (6.194)	19.44	32.00	39.60
BP-25 R Ethyl acetate	41.56 (6.447)	52.27 (7.230)	57.96 (7.613)	50.37 (7.097)	17.90	17.49	35.90
Control- Distilled water	84.14 (9.173)	89.06 (9.437)	88.98 (9.433)	87.39 (9.348)	61.00	136.00	184.60
Control -10% Methanol	84.03 (9.167)	87.98 (9.380)	87.98 (9.380)	86.66 (9.309)	59.00	109.80	174.90
Mean	43.86 (6.623)	59.06 (7.685)	64.13 (8.008)	-			
LSD (0.05)	Treatment = (0.206); Duration = (0.244); Treatment x Duration = (0.31)						

Figures in parentheses are square root transformed values
* Mean of 10 observations

Evaluation of new chemicals against *P. capsici*

Two new chemicals namely RIL-070/FI (72WP) and Ergon 44.3% (w/w) [Kresoxim methyl 500 g/L SC] were evaluated under *in vitro* and *in planta* conditions at different concentrations against *P.capsici* and *C. gloeosporioides*. RIL-070/FI (72WP) when tested at different concentrations from 10-500 ppm showed 100% mycelial inhibition at 50ppm, sporulation at 100ppm and zoospore germination at 200ppm. The average LD50 for *in vitro* inhibition was 29.23 and LD90 was 54.43ppm.

Foliar spraying of the chemical at concentrations from 100-600ppm under challenge inoculation of *P. capsici* showed that immediately after spraying of the chemical, 500 and 600ppm concentrations showed 92% and 96% inhibition of lesion development, respectively. However the effect was reduced (64.44% and 79.25%, respectively) after 10 days of application. Soil drenching of the chemical resulted in significant reduction in mortality of the plants when compared to control and 400 and 600ppm was found effective and showed 100% reduction in mortality. The *P. capsici* population was also reduced to 77.58%, but >600ppm was found to be phytotoxic.

Ergon 44.3% (w/w) [Kresoxim methyl 500 g/L SC] was tested at different concentrations from 10 -6000ppm of the product *in vitro* against *P. capsici,* showed complete inhibition of mycelial growth and sporulation at 6000ppm whereas zoospore germination was fully inhibited at 2000ppm. The average LD50 and LD90 values were 845.51ppm and 1740.71ppm, respectively. Foliar spray of the chemical followed by challenge inoculation showed inhibition of 44.83% over control. Maximum inhibition (57.12%) was observed at 5 days after spraying at a concentration of 10000ppm and was slightly reduced afterwards and almost maintained up to 20 days. Soil application of the chemical showed 50.12% mortality at 6000ppm when compared to control, while no infection or mortality could be observed at 7000 ppm.

Evaluation of new chemicals against *R. similis*

Nematicidal activity of eight chemicals *viz*., Fipronil, Thiamethexan, Acephate, Cartap hydrochloride, Quinalphos, Flubendamide, Carbosulfan, and Chloropyriphos were evaluated against the burrowing nematode, *R. similis*. Out of eight, five chemicals *viz*., Fipronil, Thiamethexan, Cartap hydrochloride, Flubendamide, and Carbosulfan showed good nematicidal activity (Fig 1.18).

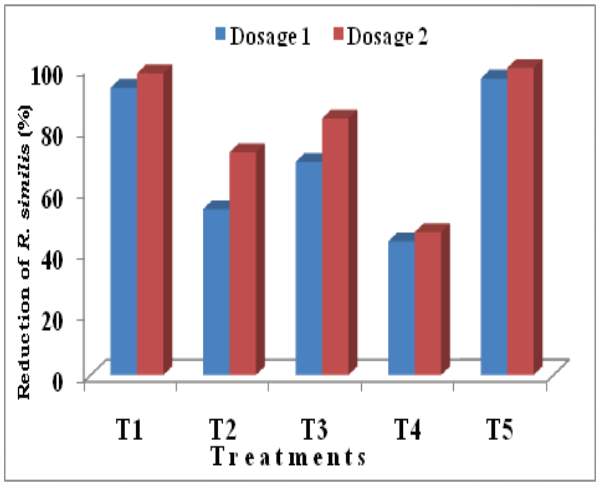


Fig 1.18 Reduction of *R. similis* population after 3 months of treatment

(T1 - Fipronil , T2 - Thiamethexan T3 - C. hydrochloride,T4 - Flubendamide ,T5 – Carbosulfan; Dosage 1- 0.005 g ai/pot, Dosage 2 – 0.01 g ai/pot)

Varietal response to biocontrol agents

Two moderately resistant lines to *P. capsici* (C1090 and IISR Shakti) and one line resistant to *R. similis* (Hp 39) were evaluated in presence of biocontrol agents *T. harzianum, P. fluorescens* (IISR 6), *P. aeruginosa* (IISR 853) and *Pochonia chlamydosporia* in comparison with susceptible vari-





ety IISR Sreekara. The establishment and growth of C1090 and IISR Shakti were highly promising in the presence of *T. harzianum* with 100% establishment without any disease incidence. Hp 39 was found to be well established in the presence of *P. chlamydosporia*. The susceptible Sreekara grew well in presence of *T. harzianum* when compared to its control where 50% plants died due to *Phytophthora* infection.

Evaluation of consortia of endophytic bacteria against *P. capsici*

Promising endophytic bacteria in different combinations were tested against *P. capsici*. The growth and establishment of the plants showed promising results with consortium containing BP 25 + BP17 and TC10 + *P. chlamydosporia* and Metalaxyl- Mz + *P. chlamydosporia*. However, on challenge inoculation Metalaxyl -Mz + BP17 showed maximum reduction in disease incidence (10%) followed by BP25 + *P. chlamydosporia* (Table 1.8).

Table 1.8 Effect of consortia on growth and disease incidence of black pepper

Treatments	Infection by *P. capsici* (%)	Root length (cm)	No. of leaves
BP25	41.90 (44.60)	14.40	7.33
BP17	42.07 (44.90)	17.40	6.67
TC10	38.73 (39.10)	7.20	7.67
BP25+BP17	44.49 (49.20)	17.38	6.17
BP25+TC10	36.90 (36.00)	11.99	7.17
P. chlamydosporia (Pc)	41.90 (44.60)	13.90	9.67
BP25+Pc	32.59 (29.00)	9.80	6.35
TC10+Pc	38.80 (39.30)	16.80	7.50
Met+Pc	36.90 (36.10)	16.77	7.33
Met+TC10	42.59 (45.80)	12.00	7.00
Met+BP17	10.00 (18.44)	9.53	13.50
Control	23.80 (16.30)	13.31	8.17
LSD (0.05)	6.53	2.99	1.39

Evaluation of actinomycetes against *P. capsici*

Nine Actinomycetes belonging to the genera *Kitasatospora* and *Streptomyces* were evaluated in the green house for disease suppression and growth promotion. The actinomycetes were grown as broth culture and inoculated to the base of the plants @100mL plant^{-1} containing a CFU of 10^8 mL^{-1}. The plants were challenged with the highly virulent isolate of *P. capsici* after one month. The results clearly revealed the efficacy of four isolates (ACt5, Act2, Act9) in checking the infection. The isolates also showed better root development and growth in rooted cuttings. The potential isolates were identified as *Kitasatospora setae* (Act2), *Streptomyces* sp. (Act5) and *S. tauricus* (Act9-VC11) (Table 1.9).

Table 1.9 Effect of different treatments on plant growth and disease incidence

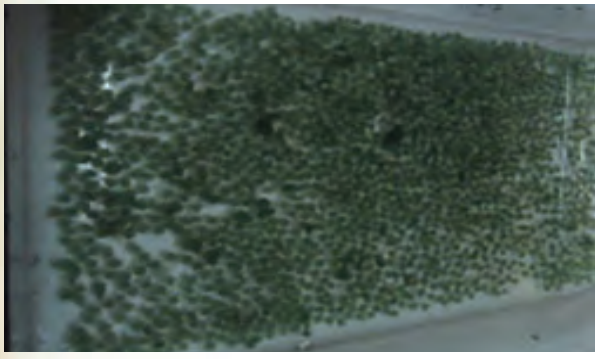
Treatments	Plant survival (%)	No of Leaves	Plant height (cm)	Root length (cm)	Root infection (%)
Act1	30	4.278	30.593	4.754	96.67
Act2	80	6.570	32.781	16.458	12.495
Act3	40	3.132	8.879	9.134	67.101
Act4	80	7.070	45.781	4.204	98.745
Act5	90	5.444	33.778	15.244	0.000
Act6	70	6.084	26.013	15.922	68.959
Act8	80	3.445	19.406	5.296	62.495
Vc11	80	3.320	11.031	10.458	12.495
Control	50	4.489	24.232	4.047	99.530
LSD (0.05)		2.268	17.687	7.071	31.356

Development of liquid formulation for biological control agents

Experiments were conducted to formulate a liquid formulation for *Trichoderma harzianum*. The viability of *T. harzianum* conidia in different cryoprotectants, effect of different storage conditions and the effectiveness of stored product for the biological control were tested. Of the different formulations tested *viz.*, Glycerol, Glucose and DMSO and sterile deionized water, mature conidial suspension in sterile deionized water was found as a promising medium for the long term storage and preservation of viable conidia of

Trichoderma with biocontrol potency for field application.

An encapsulated bead formulation of *Trichoderma* and *P. chlamydosporia* was also developed. These encapsulated formulations when mixed with vermicompost or FYM can release the organisms (Fig 1.19). The encapsulated beads can be stored in water for long term preservation. Ten encapsulated beads in 100g vermicompost or FYM gives a population of 10^{11} cfu g^{-1} in 10 days which is equivalent to or more than 10kg of talc based formulation.



(a)

(b)

Fig 1.19 Formulations of *Trichoderma* sp. a) Encapsulated beads b) Liquid formulation

Multi location testing of *Trichoderma* isolates

Two pot culture experiments were done with geographically different isolates of *Trichoderma* in varieties Panchami and Sreekara with challenge inoculation with *P. capsici* (05-06). The fungal population did not show much difference whereas there was a substantial increase in native *Trichoderma* population. The treatment T10 (PhytoFuRa10) showed minimum disease incidence (8.175%) followed by T3 (PhytoFuRa3) with (10.68%) (Table 1.10). PhytoFuRa4, 5, 6 & 7 showed more than 80% disease incidence. The treatment with PhytoFuRa13 showed maximum plant height. In case of var. Sreekara, treatment T10 showed less disease incidence followed by PhytoFuRa11, 12 &13 (less than 20% disease incidence). The treatment with PhytoFuRa2 showed maximum plant height.

Table 1.10 Evaluation of crop/pathogen specific *Trichoderma* isolates

		Disease incidence (%)	
Treatments	*Trichoderma*	Sreekara	Panchami
T1	PhytoFuRa1	22.91E	40.27CDE
T2	PhytoFuRa2	37.63CDE	55.27BCDE
T3	PhytoFuRa3	22.91E	10.68E
T4	PhytoFuRa4	80.00A	90.00A
T5	PhytoFuRa5	70.00AB	90.00A
T6	PhytoFuRa6	67.50ABC	90.00A
T7	PhytoFuRa7	57.50ABCD	80AB
T8	PhytoFuRa8	20.41E	18.04E
T9	PhytoFuRa9	60.00ABCD	60.13ABC
T10	PhytoFuRa10	10.54E	8.175A
T11	PhytoFuRa11	12.21E	30.41CDE
T12	PhytoFuRa12	37.63CDE	40.40CDE
T13	PhytoFuRa13	13.04E	25.56DE
T14	PhytoFuRa14	42.63BCDE	60.00ABC
T15	PhytoFuRa15	35.13DE	25.54DE
T16	Met-Mnz	37.77CDE	55.13BCD
T17	Control	80.00A	85AB





2. CARDAMOM





Crop Improvement

Maintenance of germplasm

Five hundred and ninety two accessions have been maintained in the NAGS at Appangala [442 accessions of Appangala (IISR), 73 accessions of Pampadumpara (KAU), 47 accessions of Mudigere (ZHRS) and 30 accessions of Sakleshpur (ICRI)] and 60 accessions have been characterized. Accession FGB46 recorded maximum yield and more number of capsules plant^{-1}. One exploration was undertaken to Sultania region in Megamalai area and 5 drought tolerant accessions were collected (Fig 2.1).


Fig 2.1 Drought tolerant accessions collected from Megamalai region

Breeding for high yield and disease resistance

Cardamom variety Appangala-1 was registered with Protection of Plant Variety and Farmers Rights Authority, New Delhi. The following five hybrids were shortlisted for Coordinated Varietal Trials (CVT) and are under multiplication.

- NKE12 x MB5 (1499kg ha^{-1})
- MB5 x NKE19 (1461kg ha^{-1})
- GG x NKE12 (1350kg ha^{-1})
- RR1 x CCS1 (1245kg ha^{-1})
- CCS1 x RR1 (1022kg ha^{-1})

Under PET 2, 3 entries *viz*., ASH (1930kg ha^{-1}), NKE-12 x GG (1746kg ha^{-1}) and GG x NKE -19 (1635kg ha^{-1}) with high yield were shortlisted for CVT.

Among the entries in the CVT, IC 349651 recorded the highest yield followed by IC 547185 (Table 2.1).

Table 2.1 Evaluation of hybrids under CVT

Genotypes	Yield (kg ha^{-1})		Mean
	2011-12	2012-13	
IC 349545	1085.33	957.87	1021.60
IC 349651	1764.00	801.25	1282.63
IC 547167	1393.12	716.80	1054.96
IC 547185	1449.60	870.34	1159.97
CL 691	606.30	380.34	493.32
CL 726	593.93	321.46	457.70
PL NO. 14	1042.92	648.67	845.80
CR 6	419.50	297.43	358.47
MCC 346	521.55	461.36	491.46
SKP 104	718.10	398.67	558.39
SKP 164	852.60	423.35	637.98
Mean	949.72	570.69	760.20
CV	46.00	41.34	42.57
CD(0.05)	391.23	280.04	

Screening hybrids for *Katte* resistance

Hybrids were established under greenhouse to screen against *Katte* disease. 300 F2 mapping population from the cross between NKE 12 x GG were artificially inoculated with *Katte* virus under green house conditions and 240 were found to be susceptible after 3rd round of inoculation. Among 230 F2 mapping population (GG x NKE 12), 45 were found susceptible after first round of aphid inoculation.

Screening hybrids for resistance to thrips

Twenty hybrids from the germplasm block at CRC Appangala were field screened against thrips. Total number of capsules, the total number of capsules damaged, numbers of cardamom capsules with low (<25%), medium (>25% - <50%) and heavy (>50%) thrips attack were recorded. The lowest total capsule damage was recorded on GG x NKE19 cross (27.38%), whereas, the hybrid RR1 x CCS1 recorded the highest total capsule damage of 91.3 %. All the hybrids were found susceptible to thrips.

Screening germplasm for abiotic stress

Physiological parameters such as relative water content (RWC), specific leaf weight and stomatal count were recorded in 30 accessions. Significant variation was recorded for RWC and specific leaf weight.

Screening germplasm for biotic stress

Natural incidence of leaf blight and rhizome rot diseases were recorded in 60 accessions maintained in the field gene bank (FGB). The accessions were further grouped into different categories based on the reaction towards leaf blight (Table 2.2) and rhizome rot disease (Table 2.3).

Table 2.2 Reaction of field gene bank accessions to leaf blight

Percent Disease Index	Category	Accessions
< 10	Highly resistant (HR)	Nil
11 – 20	Resistant (R)	FGB 1, FGB 2, FGB 3, FGB 4, FGB 5, FGB 7, FGB 8, FGB 9, FGB 11, FGB 13, FGB 14, FGB 15, FGB 18, FGB 19, FGB 21, FGB 22, FGB 24, FGB 25, FGB 27, FGB 28, FGB 30, FGB 31, FGB 37, FGB 39, FGB 44, FGB 46, FGB 52, FGB 53, FGB 55, FGB 56, FGB 58, FGB 60
21 – 30	Moderately resistant (MR)	FGB 6, FGB 10, FGB 12, FGB 16, FGB 17, FGB 20, FGB 23, FGB 26, FGB 29, FGB 32, FGB 33, FGB 34, FGB 35, FGB 36, FGB 41, FGB 45, FGB 47, FGB 48, FGB 50, FGB 54, FGB 57, FGB 59
31 – 40	Moderately susceptible (MS)	FGB 38, FGB 40, FGB 42, FGB 43, FGB 49, FGB 51
41 – 50	Susceptible (S)	Nil
> 51	Highly susceptible (HS)	Nil

Table 2.3 Reaction of field gene bank accessions to rhizome rot

Percent Disease Index	Category	Accessions
0.0 – 5.0	Highly resistant (HR)	FGB 1, FGB 3, FGB 8, FGB 9, FGB 13, FGB 21, FGB 22, FGB 26, FGB 28, FGB 29, FGB 30, FGB 45, FGB 52, FGB 60
5.1 – 10.0	Resistant (R)	FGB 5, FGB 19, FGB 27, FGB 31, FGB 33, FGB 34, FGB 49, FGB 50, FGB 58
10.1 – 25.0	Moderately susceptible (MS)	FGB 2, FGB 4, FGB 6, FGB 7, FGB 10, FGB 11, FGB 12, FGB 14, FGB 15, FGB 16, FGB 17, FGB 18, FGB 20, FGB 23, FGB 25, FGB 32, FGB 35, FGB 44, FGB 46, FGB 47, FGB 48, FGB 51, FGB 53, FGB 54, FGB 55, FGB 56, FGB 57, FGB 59
25.1 – 50.0	Susceptible (S)	FGB 24, FGB 36, FGB 37, FGB 38, FGB 41, FGB 42, FGB 43
> 50	Highly susceptible (HS)	FGB 39, FGB 40





Crop Production

Quantifying nutrient removal for targeted yield





To find out the nutrient requirement for targeted yield, graded fertilizer treatments were imposed with four levels – T1- 0,0,0 (control); T2- 75,75,150 T3- 125,125,250 and T4- 150,150,300 kg NPK on two varieties of cardamom (Appangala-1 - Paka estate and Green Gold - Kauvery estate). In Appangala-1, higher yields were recorded for higher fertilizer levels with the highest in T4 (824kg ha^{-1}) followed by T3 (740kg ha^{-1}), T2 (683kg ha^{-1}) and lowest in control (209kg ha^{-1}). Number of yielding tillers, no. of panicles $clump^{-1}$ and no. of capsules $panicle^{-1}$ were significantly higher in fertilizer treatments compared to control. In Green gold also highest yield was recorded in T4 (883kg ha^{-1}) where highest dose was applied followed by T3 (795kg ha^{-1}) and T2 (739kg ha^{-1}) with the lowest in the control (344kg ha^{-1}). The fertilizer treatments recorded significantly higher no. of panicles $clump^{-1}$ and no. of capsules $panicle^{-1}$ as compared to control. The average weight of dry matter removed by thrashing (dried tillers, leaves and panicle) during a year and the nutrient removal by this dry matter were quantified. The nutrient removal by the capsule was quantified as 6.1, 1.9 and 4.2kg of N, P_2O_5 and K_2O by 100kg of the produce. From these estimates, the nutrient required for production of 100kg of capsule has been worked out as 11.3, 1.6, 9.1kg of N, P_2O_5 and K_2O by Green gold and 12.2, 1.7, 11.9kg of N, P_2O_5 and K_2O by Appangala-1.

Developing electronic nose for monitoring aroma

This project aims at developing electronic nose for monitoring the quality of cardamom. Essential oil content and composition of 11 genotypes were determined and the Normaroma index was determined using *e*-nose, developed for assessing the quality of tea. In the preliminary studies, quantity of sample (cardamom seeds 5g) and size of sample holder (10g) were standardized (Table 2.4).

Table 2.4 Normaroma index from alpha-MOS

Capacity of sample holder	Normaroma index			
	Whole cardamom (5g)		Cardamom seeds (5g)	
	R 1	R 2	R 1	R 2
250g	14.1	20.2	54.4	56.1
	10.6	15.0	53.0	59.2
125g	4.2	5.3	27.8	29.0
	4.3	4.0	31.5	35.9
10g	9.0	8.7	33.3	35.7
	7.0	6.2	3.0	36.0

Crop Protection

Evaluation for thrips resistance

Two hundred and ninety six accessions were screened against thrips. There was wide variation in the reaction of the accessions to thrips and percentage of infested capsules ranged from 3.0-96.2%. Fifty three accessions recorded <20% capsule damage and 5 accessions recorded <10% capsule damage. Four of the 5 accessions with <10% capsule damage belonged to *Malabar* types and one to *Vazhukka* type. All the 9 highly susceptible accessions belonged to either *Mysore* or *Vazhukka* types.

Plant morphological characters such as nature of leaf sheath (loose or tight), flower bract (persistent or non-persistent) and panicle type (erect, semi erect or prostrate) were recorded in 69 accessions for characterization against thrips

attack. The mean capsule damage was higher (57.2%) in *Mysore* types compared to *Malabar* types (32.8%). Plant characters such as erect panicle and persistence of flower bract contributed to higher thrips incidence.

Evaluation of insecticides and organic products for thrips control

Eleven insecticide molecules and organic products *viz.,* neem soap (10g L^{-1}), Spinosad (0.3mL L^{-1}), Vertemec (0.4mL L^{-1}), Thiamethoxam (0.3mL L^{-1}), Thiacloprid (0.5mL L^{-1}), Imidacloprid (0.5mL L^{-1}), L-cyhalothrin (0.5mL L^{-1}), Zolone (2.0mL L^{-1}), Fipronil (1.0mL L^{-1}) and Quinalphos (2.0mL L^{-1}) were evaluated for their efficacy in management of thrips. The sprays were imposed during March, April, May, August and September. The trial indicated that Fipronil was the most effective in controlling the thrips population throughout the cropping season followed by Imidacloprid, Quinalphos, Thiacloprid and Thiamethoxam which were on par with each other.

Documentation of endosymbionts and entomopathogens on thrips

The bacterial endosymbiont *Wolbachia* was identified from thrips collected from Kodagu (Karnataka), Wayanad, Palakkad, Idukki (Kerala), Yercaud, Nilgiris and Kodaikanal (Tamil Nadu) districts. All the thrips populations were infected with only sub group Con belonging to super group B. Both male and female thrips were infected with the same *Wolbachia* subgroup. An entomopathogenic fungus was isolated from thrips during a survey of cardamom plantations in Wayanad District in Kerala.

Diversity of rhizome- root rot pathogens and their antagonists

Surveys were repeated in Wayanad and Idukki districts of Kerala, Hassan and Kodagu districts of Karnataka to study the seasonal variation of rhizome and root rot diseases. Eighty fungal isolates were isolated from rhizome and root rot disease affected samples. Among the isolates, *Rhizoctonia solani, Pythium vexans* and *Fusarium* species were found to be dominant. Artificial inoculation studies proved that among the different fungi isolated, *R. solani, P. vexans* and *Fusarium oxysporum* were pathogenic.

Different species of *Fusarium* were found associated with root rot disease, of which *F. oxysporum* was found to be the predominant species. Morphological characterization revealed the association of different species *viz., F. oxysporum, F. solani, F. pallidoroseum* and *F. verticillioides. Rhizoctonia* isolates from all the locations surveyed were identified as *R. solani*.

Protocols for DNA isolation of *F. oxysporum* and *Trichoderma* have been standardized. *In vitro* screening of respective *Trichoderma* spp. against the most virulent root rot pathogen, *F. oxysporum* isolates from Kerala, Karnataka and Tamil Nadu led to the identification of WYDT6, RT 7B and RT2A, respectively as the most effective isolates (Fig 2.2).

Fig 2.2 WYDT6, an effective isolate collected from Kerala









Endophytic and rhizospheric microflora

Isolations made from different plant parts including leaves, petioles, pseudostem, roots and rhizomes of *Amomum* sp. and *Alpinia* sp. yielded 50 fungal isolates and 5 bacterial isolates. Among the plant parts, the isolates had more preference towards pseudostem (Fig 2.3) followed by rhizomes and least for the roots. Endophytic fungi and bacteria were also isolated from 3 ecotypes *viz., Malabar, Mysore* and *Vazhukka*.

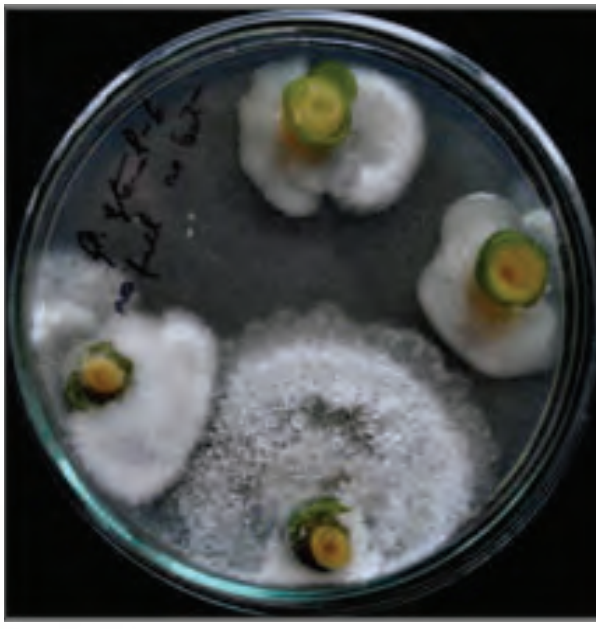


Fig 2.3 Fungal endophytes isolated from pseudostem

Studies on mycelial compatibility groups among isolates of *Colletotrichum gloeosporioides*

Mycelial compatibility studies among the selected isolates of *C. gloeosporioides* (3 each from Kerala and Tamil Nadu and 8 isolates from Karnataka) revealed that majority of the isolates showed incompatible reaction when isolates from different geographical locations were paired, while, isolates from same geographical regions exhibited compatible reaction when paired together.

IDM strategy for nursery

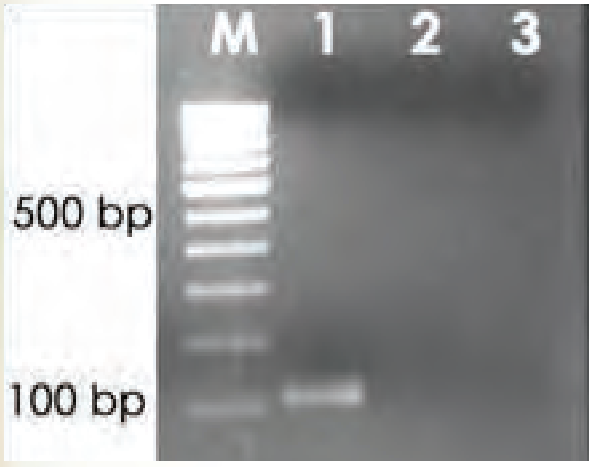
Evaluation of fungicides, a neem based product and an isolate of *T. harzianum* revealed that spraying the combination product of carbendazim + mancozeb in combination with soil application of *T. harzianum* was promising in managing leaf spot disease under nursery conditions (Fig 2.4).

Fig 2.4 Management of leaf spot under cardamom nursery

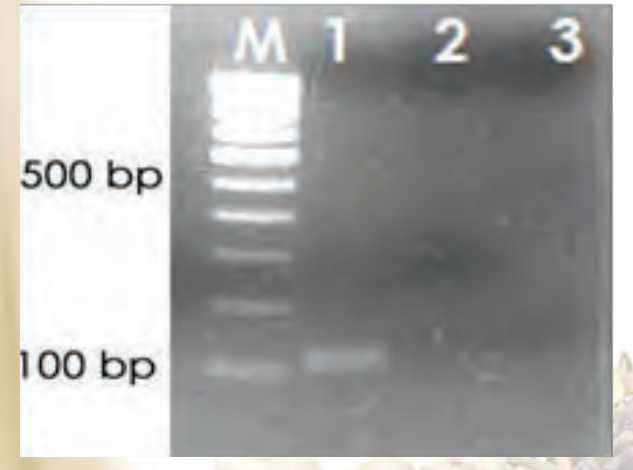
Real time RT-PCR for detection of viruses

A method for the detection of Cardamom mosaic virus (CdMV) and Banana bract mosaic virus (BBrMV) was developed with the use of SYBR green one step RT-PCR. Primers were designed from conserved coat protein gene sequences available in GenBank database to amplify 127 bp and 117 bp fragments of CdMV and BBrMV, respectively. The method involved isolation of total RNA and subjecting to real time RT-PCR using qPCR master mix, specific primers and reverse transcriptase for each of the virus. The assay amplified all the isolates of CdMV and BBrMV but no amplification was obtained with the RNA of healthy host plants. For SYBR Green based virus detection, it is important to run a melt curve analysis followed by real time PCR, because SYBR Green real time RT-PCR detects each double stranded DNA including primer dimmers and PCR products from mis-annealed primer. Examining the melt

curve ensures that the desired amplicon was detected. The melting temperature of the amplicon starts at the point of inflection of the melt curve profile. The melt peak occurs at 83˚C±0.5 for CdMV and BBrMV and no evidence of nonspecific amplification or primer-dimerization. The healthy and water control showed no melting peak. The specificity of the product was further confirmed by agarose gel electrophoresis which showed a single band of the expected size (Fig. 2.5).



(A)



(B)

Fig 2.5 Agarose gel electrophoresis of Reverse transcription quantitative real time PCR (RT-qPCR) for the detection of (A) Cardamom mosaic virus (B) Banana bract mosaic virus Lane 1: Infected cardamom; Lane 2: healthy cardamom; Lane 3: water control. Lane M shows 100 bp DNA ladder.

Recombinant plasmids carrying target virus regions corresponding to both the viruses were quantified, serially diluted and used as standards for real time PCR to develop standard curve to enable quantification. The amplification of the standard dilution series yielded in linear relations with reliable results (R^2 = 0.99906 for CdMV and R^2 = 0.99902 for BBrMV) in the range of 10–10^8 copies of the target DNA locus per PCR. The detection limit for CdMV was as low as 23 copies and 24 copies for BBrMV as estimated from the standard curve. Similarly the detection limits of the viruses in plants were also studied by using 10 fold dilutions of RNA from infected cardamom plant (1 to 10^{-5}). By taking the Ct values for each dilution the detection limit (copy number) of CdMV and BBrMV was determined from the standard curve. It was found that the detection limit is as low as 16 and 10 copies (10^{-4} dilution) for CdMV and BBrMV respectively, which was approximately 1000 fold higher than conventional RT-PCR. The RT-qPCR method for CdMV and BBrMV were further validated by testing 30 CdMV and 20 BBrMV isolates from Kerala and Karnataka states. The new method offers a fast, reliable, specific and sensitive tool for the detection of CdMV and BBrMV in cardamom.

Development of RT-LAMP for detection of virus

A reverse transcription loop-mediated isothermal amplification (RT-LAMP) method was developed for quick detection of Banana bract mosaic virus (BBrMV). Five sets of primers were designed based on conserved sequences in coat protein gene of the virus. The RT-LAMP reaction components were optimized by using total RNA extracted from a known infected plant (positive control), a known healthy plant (negative control) and water control (negative control containing water instead of RNA). The LAMP reactions were then carried out at 65°C for 75 min. For the visualization of RT-LAMP products, three different methods were used. During LAMP reaction a large amount of DNA is synthesized, which yields a large amount of pyrophosphate ion by-product





(magnesium pyrophosphate) which is insoluble thus increasing turbidity of the reaction tube which can be visualized by different means. In the first method, the presence or absence of turbidity in the reaction tube was taken as the criteria for detection. In the second method, addition of 1mM $MnCl_2$ and 50µM calcein to the reaction mix resulted in the formation of a fluorescent metal indicator which can be viewed under UV light. In the third method, amplification products were visualized by 2% agarose gel electrophoresis. Presence of turbidity or green florescence allowed easy detection of RT-LAMP products without gel electrophoresis. Detection limit of RT-LAMP was up to 100 times higher than RT-PCR and on par with real time RT-PCR. In order to validate the method, total RNA isolated from 24 test samples of cardamom and banana collected from Karnataka and Kerala were subjected to RT-LAMP using 1µL of template RNA along with known positive and negative controls. Of the 24 samples tested, 22 samples showed positive reaction, indicating successful detection of BBrMV in different isolates of virus.





3. GINGER





Crop Improvement

Genetic resources

Six hundred and sixty eight *Zingiber* accessions have been conserved under field gene bank. Germplasm conservatory was enriched with 36 accessions from RAU, Dholi, Bihar. Based on request and under MTA, 12 accessions were shared with five research organizations.

Evaluation of promising nematode resistant accessions

Promising nematode tolerant accessions were evaluated from 2010 to 2013 for yield. Among 4 accessions, maximum yield was recorded in Acc. 219 followed by IISR Varada (Table 3.1).

Table 3.1 Evaluation of nematode tolerant ginger accessions

Entries	Fresh yield (kg $3m^{-2}$)				
	2010-11	2011-12	2012-13	Mean	t ha^{-1}
Acc. 372	6.67	3.99	7.50	6.05	13.31
Acc. 65	7.33	4.03	10.00	7.12	15.66
Acc. 219	11.00	5.64	10.00	8.88	19.54
Acc. 251	8.67	3.96	8.50	7.04	15.49
Varada	10.63	5.07	8.83	8.18	18.00
Mahima	7.27	4.93	9.23	7.14	15.71
Mean	8.59	4.60	9.01	7.40	
CD (0.05)	1.33	1.24	1.19	0.62	
CV (%)	10.24	14.76	8.74	10.17	

Visual selection for boldness was imposed on the germplasm and 13 lines were selected for its extra bold rhizome character. These lines were further multiplied to generate planting material for yield and quality assessment. Breeder's seeds of IISR released varieties were planted for multiplication.

Induction of variability through induced mutation

The rhizome buds of 3 varieties (IISR Mahima, IISR Varada, IISR Rejatha) were subjected to Y irradiation at different doses of 0.5, 0.75, 1.0, 1.25 and 1.50 kR (1kR = 1000 rad) with a control. Based on probit analysis for mortality percent in Y irradiated ginger, LD50 for 3 varieties was derived. Total of 5.13% chlorophyll mutation frequency was recorded among M1V1 mutants (Fig 3.1).

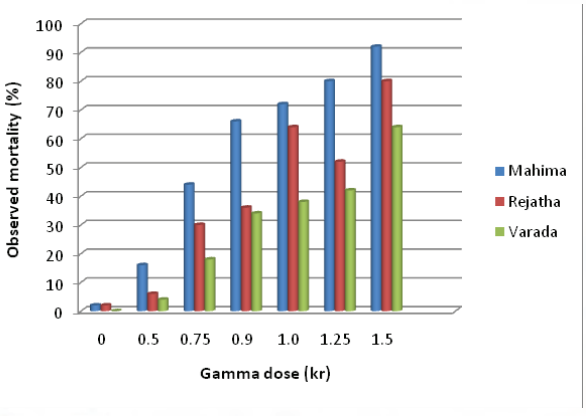


Fig 3.1 Mortality (%) and LD50 dosage

Ten shoot bud cultures each of 3 cultivars *viz.*, IISR Varada, IISR Rejatha and IISR Mahima were established *in vitro*. These will be further utilized for establishing callus cultures for *in vitro* mutagenesis. Four *R. solanacearum* resistant mutants were planted for multiplication to take up further yield evaluation. 177 (M1V6) and 87 (M1V7) mutants were maintained in pots. 128 mutants (M1V7 and M1V6) were subjected to preliminary screening for soft rot disease caused





by *P. myriotylum* and 35 mutants which escaped infection were short listed. These mutants will be multiplied and subjected to secondary screening during the next season.

Transcriptomics

In order to achieve a broad survey of genes associated with bacterial wilt resistance, mRNA-Seq profiling of *C. amada* and *Z. officinale* leaves following infection with *R. solanacearum* was performed. Leaf samples were collected pooled within a given time point, mRNA was isolated, enriched, sheared into smaller fragments, and reverse-transcribed into cDNA. The cDNA were subjected to Illumina HiSeq™ 2000 sequencing, and the resulting sequencing data were subjected to bioinformatic analysis. A total of 31,845,321x2 (101 base), 24,107,482x2 (101 base) raw reads were generated, accounting for approximately 6.43 Gb and 4.87Gb of sequence data, for *C. amada* and *Z. officinale*, respectively. The contigs were assembled into transcripts using the transcriptome assembly software Bowtie. Transcripts which were shorter than 200 bases in length were filtered out, resulting in 45045 and 65536 transcripts from the *C. amada* and *Z. officinale* libraries, respectively. The transcriptomes of ginger and mango ginger following infection by *R. solanacearum* were compared, and several candidate genes for resistance to bacterial wilt were identified. (Fig 3.2).

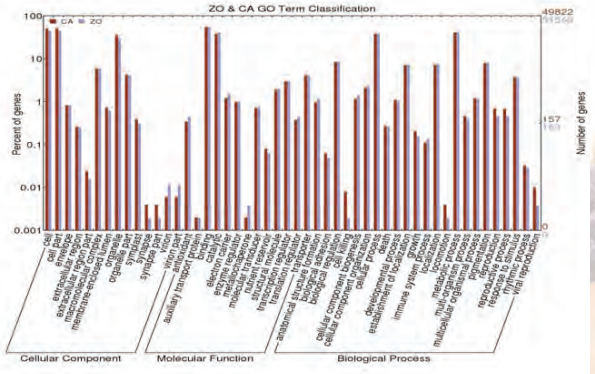


Fig 3.2 Histogram of Gene Ontology classification of putative molecular function of unigenes from mango ginger and ginger tissues and biological processes in which they are involved

Suppression subtractive hybridization

RNA samples derived from plants after 4 and 8h after inoculation (hai) were used to understand the early genes involved in bacterial wit resistance in *C.amada* by subtractive hybridization. Clones containing differentially expressed cDNAs of *C.amada* were sequenced using M13 forward and reverse primers. Of the 150 clones, sequenced, 138 produced suitable sequences and were evaluated by computational analysis. All the sequences were annotated using the PSI-BLAST program. Of the 138 SSH sequences, 15 had no identity with sequences in the databases. Among these differentially expressed genes, sequences coding for putative proteins related to defense response during *C.amada—R.solanacearum* interaction (high homology between 85 and 100% identity) were, glutathione-S-transferase (GST), putative cytosolic ascorbate peroxidase (APX), cell wall associated hydrolase, Xyloglucan transglycosylase (XTH), cytochrome p 450, metallocarboxypeptidase inhibitor, peroxiredoxin and thioredoxin dependent peroxidase.

Crop Production

Organic farming

An organic package consisting of 20t FYM + 2t neem cake + 1t ash + 4t vermicompost ha^{-1}, *Azospirillum* and P Solubilising bacteria (20g bed^{-1}) for nutrient supplement, GRB35 as seed treatment and application of Panchagavya as drench and spray at 45 and 90 DAP has been standardized. Drenching of GRB35 or 57 and GEB17 was helpful in keeping the disease incidence to <10-15%. Spray of BM (1%) to contain foliar diseases and neem oil (5mL L^{-1} of water) in combination with the cultural control is recommended for shoot borer management. Results showed that organic management system yielded on par with integrated system, whereas the quality was found to be superior under organic systems.

PGPR as biocontrol

The rhizobacterial strain, GRB35 confirmed its plant growth promoting efficiency besides reducing the soft rot and bacterial wilt in ginger (var. Mahima). The field experiment consisted of treatments involving GRB35 delivered through various modes The data (Fig 3.4) revealed that among the treatments, activated capsule (2 capsule $5kg^{-1}$ seed)-T2 followed by non activated capsule (1 capsule $5kg^{-1}$ seed)-T1 registered markedly lower soft rot incidence (11 and 12%, respectively).

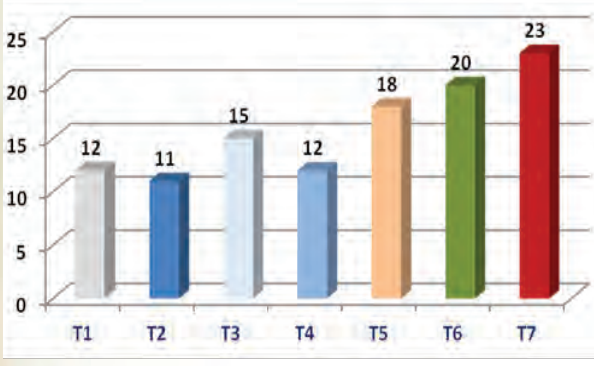


Fig 3.4 Effects of different modes of PGPR delivery on soft rot incidence (%) in ginger (T1- Non activated capsule, 1 per 5 kg seed; T2- Activated capsule, 2 per 5 kg seed; T3- Activated capsule, 1 per 10 kg seed; T4- Activated capsule, 1per 5 kg seed; T5- Talc formulation, 10 g 10 L-1; T6- Metalaxyl- Mancozeb, 1.25g L-1; T7- Absolute control)

Source- sink relationship

Varieties IISR Varada, IISR Rejatha and IISR Mahima were planted and sampling was done at monthly interval starting from 50 days after planting (DAP). Plant height, number of leaves, leaf area and dry weight were recorded at monthly intervals starting from 50 DAP. IISR Varada accumulated more leaf area and dry weight at all sampling dates.

Starch content increased steadily from 50 to 110 DAP and then there was sudden rise in all the varieties. IISR Varada showed highest starch content. Dry matter partitioning studies showed that up to 80 DAP, shoot had more biomass than rhizomes and at 110 DAP, rhizome had more biomass compared to shoot. At 150 DAP, rhizomes accumulated >75% biomass in all the varieties.

Endogenous IAA and zeatin riboside levels were quantified in both leaves and rhizomes. Rhizomes and leaves recorded similar levels and the maximum levels at 80 DAP (Table 3.2). IISR Varada had slightly higher levels. All varieties showed higher photosynthetic rate at 120 DAP compared to 90 DAP and all the varieties had similar levels of photosynthesis. Photosynthetic rate ranged from 10.4-13.9µmoles.

Table 3.2 Starch and plant hormones in different varieties

Days after planting	Rhizome starch (%)	IAA (µ moles)	Zeatin riboside (µ moles)
IISR Rejatha			
50	5.6	22.6	14.4
80	10.2	45.4	33.7
110	16.7	40.1	26.1
150	40.0		
IISR Mahima			
50	6.2	19.4	12.2
80	9.7	46.5	28.5
110	14.8	38.1	24.7
150	38.8		
IISR Varada			
50	6.6	21.5	14.8
80	11.4	52.0	35.2
110	17.3	43.8	30.3
150	41.4		

Weed management practices on growth, yield and quality

An experiment was initiated to study effect of weed management practices on weed control efficiency and to study growth, yield, quality parameters and disease incidence with 10 different green and polythene mulch combinations. Height, number of tiller production and yield were higher for the treatments with *Glycosmis pentaphylla* leaves (recommended practices), *Lantana camara* leaves, paddy straw, coir pith compost and application of coconut leaves as mulch in the beds. Maximum weed control efficiency (97%) was recorded by spreading of black colour polythene mulch followed by application





of ash colour polythene mulch. Weed control efficiency was also higher in the treatment involving coir pith compost (94%) followed by coconut leaves. Shoot borer incidence varied from 10-20% and maximum was observed in beds in which coir pith compost was applied. Percentage of clump infected with *Rhizoctonia* was more in the treatment in which ash colour polythene mulch was used.





Crop Protection

Isolation and characterization of phages infecting *R. solanacearum*

Rhizosphere soil samples were collected from ginger growing areas of Kerala and Karnataka and phages were isolated, using *R. solanacearum* isolate from Wayanad as the host. The host range of this phage was checked using seventeen different isolates of *R. solanacearum* (Fig 3.5). This phage was found to be infecting all the isolates collected from Wayanad. The phage was PEG purified and titrated. The stock was found to contain 10^7 pfu mL^{-1}.

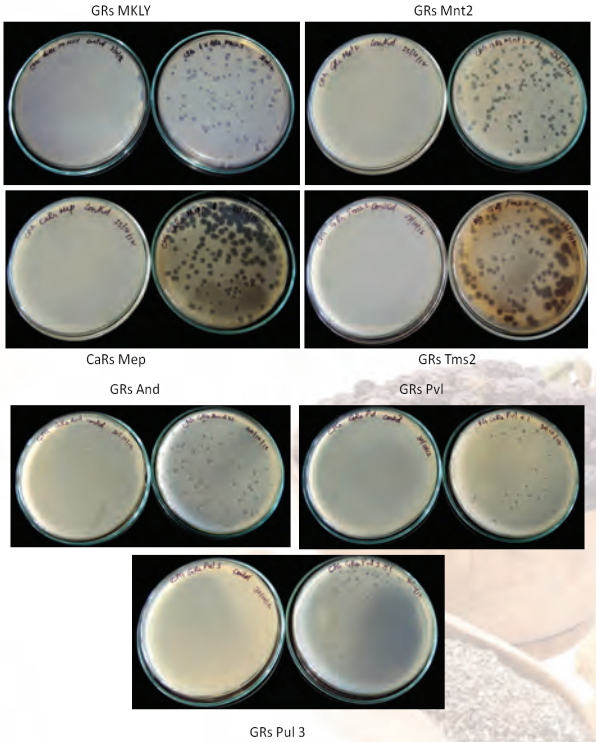


Fig. 3.5 Host range of the isolated phage

The phage DNA attP site was amplified using the primer pairs attP - L 5'-CAGTATGTGTCCTGGGTGTTTGTCTACCG-3' and attP - R 5'- CTCTTATCAGAACGCCCCACCTCCC-3'. But there was no amplification indicating the absence of attP region. So this phage can be effectively utilized for the biocontrol of *R. solanacearum*.

Isolation of bacteria from apoplastic fluid

The pseudostem and leaves were used for the apoplastic fluid extraction. The apoplastic fluid obtained was serially diluted and plated on tryptic soy agar medium. The distinct colonies obtained were purified and stored. Hundred and fifty bacterial isolates were maintained as glycerol stocks. These bacteria will be checked for their biocontrol properties and will be shortlisted for pot culture and field evaluation.

Volatile oil and 3 solvent extracts (hexane, methanol and chloroform) from the rhizome of *C. amada* were used to examine the antimicrobial activity *in vitro*. The extracts showed varying levels of inhibitory effect on the test pathogens. Among the solvent extracts chloroform extract exhibited greatest antibacterial activity against *R. solanacearum*, followed by hexane and methanol extracts. In case of *P. myriotylum*, hexane extracts showed greatest activity followed by chloroform extract. Methanol extract exhibited least effect. Essential oil at 1.5% (v/v) caused the complete death of bacteria showing greatest antimicrobial effect. Essential oil was subjected to GC-MS analysis and the major components detected were β -Myrcene and β -Pinene. These were tested for their inhibitory activity against *R. solanacearum*. Both the components showed antibacterial activity with myrcene showing greater activity.

Biochemical characterization of resistant and susceptible accessions to shoot borer *(Conogethes punctiferalis)*

Total fibre and lignin contents in mature shoots and total carbohydrates, lignin and proteins in mature leaves were estimated in mod-

erately resistant and susceptible accessions to shoot borer. Lignin, total proteins, phenols, carbohydrates and fibre contents in immature leaf tissues and immature shoots were also determined

Table 3.3 Biochemical characterization of moderately resistant and susceptible accessions

Character	Mature				Tender			
	Leaf		Shoot		Leaf		Shoot	
	MR	S	MR	S	MR	S	MR	S
Phenol (mg 100mg^{-1})	-	-	-	-	0.24–0.49	0.31–0.613	0.16– 0.44	0.13– 0.19
Carbohydrate (mg 100mg^{-1})	6.22–13.05	6.86–16.31	-	-	-	-	-	-
Protein (mg 100mg^{-1})	1.47–4.61	1.28–2.72	-	-	-	-	-	-
Lignin (%)	10.05–18.94	11.13–18.08	8.59-17.60	17.09-17.06	27.13-41.05	29.91-42.26	35.43-43.03	42.56-43.47
Fibre (%)	-	-	24.60–34.30	19.30-27.70	21.18-24.02	22.97–26.72	22.6–32.6	22.0- 27.9
Epicuticular wax (mg 75cm^{-2})	-	-	-	-	20.00-27.00	18.00-27.00	-	-

MR = moderately resistant; S = susceptible

in susceptible as well as in moderately resistant accessions. Epicuticular wax was estimated only in immature leaf tissues (Table 3.3)

Evaluation of EPNs

The infectivity of 4 promising EPNs *viz., Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius gingeri* and *Oscheius* sp. (IISR-EPN 08) was tested against shoot borer larva in the greenhouse. The treatments included application of the EPNs @ 25000Ijs pot^{-1} at 21 days interval during August to October, spraying of Malathion 0.1% (present recommendation) and control. Shoot damage was recorded at the end of crop season. Among the EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* treated plants showed minimum shoot damage (15.8% and 16.6%, respectively) which was significantly superior over control (36.0%) and was on par with insecticide application.





4. TURMERIC





Crop Improvement

Genetic resources

One thousand three hundred forty two *Curcuma* accessions have been conserved in the field gene bank. Germplasm conservatory was enriched with 92 accessions received from RAU, Dholi, Bihar and TNAU, Coimbatore, Tamil Nadu. Based on request and under MTA, 20 accessions were shared with 5 research organizations.

Morphological characters including the plant and rhizome characters were recorded in 87 accessions as per the DUS guidelines.

Yield evaluation of promising accessions

Promising nematode tolerant accessions were evaluated for yield. The different accessions varied in height and rhizome characteristics. Maximum mean yield over 3 years was recorded in Accessions 48 and 49, followed by IISR Prathibha (Table 4.1)

Table 4.1. Evaluation of nematode tolerant accessions for yield

Entries	Fresh yield (kg $3m^{-2}$)				
	2010-11	2011-12	2012-13	Mean	t ha^{-1}
Acc. 200	10.50	9.67	10.38	10.81	23.78
Acc. 142	9.75	11.42	14.56	11.91	26.20
Acc. 79	14.00	13.34	16.00	14.45	31.79
Acc. 35	12.00	9.92	15.25	12.39	27.26
Acc. 48	14.00	13.44	16.13	14.52	31.94
Acc. 146	10.25	12.32	14.88	12.48	27.46
Acc. 130	9.75	9.05	12.88	10.56	23.23
Acc. 376	10.25	10.42	12.75	11.14	24.51
Prathibha	15.23	10.62	12.25	12.70	27.94
Mean	11.75	11.13	13.90	12.26	
CD (0.05)	1.82	1.69	2.53	1.07	
CV (%)	10.62	8.80	12.48	10.74	

Characterization of *Curcuma species*

Four Curcuma species *viz., C. aromatica, C. caesia, C. zedoaria* and *C. amada* were characterized for agronomic and quality traits at different growth stages *viz*., 90, 140 and 180 days after planting (Tables 4.2). Preliminary data indicated the differential behavior of the different species at the 2 growth stages with respect to biochemical profile.

Table 4.2 Biochemical features of *Curcuma* species at different stages of growth

Species	Rhizome oil (%)		Starch (%)		Protein (%)		Curcumin (%)		Fiber (%)	
	90d	140d	90d	140d	90d	140d	90d	140d	90d	140d
C. amada	4.42±0.43	3.4±0.20	18.01±1.98	35.1±1.17	10.56±0.84	9.35±0.61	0.06±0.03	0.04±0.02	2.8±0.23	2.82±0.09
C. aromatica	6.97±0.13	5.9±0.30	37.8±7.77	37.2±1.32	8.52±1.21	8.25±0.53	0.03±0.01	0.04±0.00	5.9±0.30	3.26±0.24
C. caesia	4.75±0.25	3.4±0.20	51.25±2.41	42.95±1.46	9.4±0.51	8.32±0.32	0.09±0.03	0.06±0.01	3.75±0.75	3.15±0.13
C. xanthorrhiza	1.9±0.10	3.2±0.16	44.95±4.33	43.4±1.54	8.38±0.82	7.85±0.39	0.10±0.01	0.16±0.01	4.05±0.41	3.08±0.15

Maintenance of seedling progenies

A total of 252 seedling progenies of 23 different mother genotypes were maintained. Sixty six second generation seedling progenies of 6 seedling progenies were also maintained. Additionally, 150 second generation seedling progenies are also being maintained. Seeds were collected from 35 seedling progenies and sown for germination and so far a total number of 225 seedlings have emerged.

Evaluation of shortlisted seedling progenies

Thirty five seedling progenies shortlisted based on the performance in the earlier trials were planted with 5 released varieties as control. Highest fresh yield of 12.09kg bed^{-1} was recorded in released variety Rajendra Sonia, followed by Prabha (10.42) and Sudarsana (10.13). Among the

seedling progenies, SLP 414/3 recorded the highest yield of 9.67kg bed^{-1}. High curcumin line SLP 389/1 produced 8.17kg bed^{-1}.

Rhizomes from 35 shortlisted seedling progenies and 2 controls harvested from the field trial showed dry recovery of >20% for most of the entries. Four of them showed dry recovery of >24%. Highest curcumin content of 5.99% and oleoresin content of 14.19% was recorded in SLP 389/1 while oil content of 4.9% was recorded in SLP 415/3. Curcumin content in controls *viz.*, Prabha, Kedaram was 4.20% and 5.07% respectively. Nine of the shortlisted progenies were also planted at CRC, Appangala to observe performance at high altitudes with released variety IISR Prathibha as control. IISR Prathibha yielded 20kg fresh rhizomes bed^{-1}. All the seedling progenies tested registered fresh yield above 8kg bed^{-1}. Seedling progeny SLP 138/78 showed 10.33kg bed^{-1}. High curcumin line 389/1 produced 9.33kg bed^{-1} (Table 4.3). Highest dry recovery of 23.17% was recorded in SLP 138/43 followed by 138/78 (19.9). High curcumin line 389/1 showed a dry recovery of 18.82% and control IISR Prathibha showed 18.28%.

Table 4.3 Yield and dry recovery of seedling progenies

Genotype	Yield* (kg bed^{-1})	Dry recovery (%)*
126/1	9.47	18.02
126/5	8.17	19.17
138/22	9.83	19.28
138/32	8.93	19.67
138/43	9.70	23.17
138/78	10.33	19.9
389/1	9.33	18.82
415/3	8.97	18.4
449/6	8.13	18.83
IISR Prathibha	20.23	18.28

* Mean of 3 replications

Highest curcumin content of 5.02 % was recorded in SLP 389/1 which was on par with the control IISR Prathibha (4.79%). All other progenies showed curcumin content <4% only (Table 4.4). The last 3 years data revealed that curcumin content in seedling progeny No. 389/1 is constantly above 5%.

Table 4.4 Quality analysis of shortlisted seedling progenies

Genotype	Dry recovery (%)	Curcumin (%)	Oleoresin (%)	Oil (%)
IISR Prathibha	17.00	4.79	11.42	3.69
389/1	19.70	5.02	13.41	4.71
449/6	19.13	3.08	11.03	4.93
415/3	20.13	2.27	10.89	4.53
138/20	20.23	2.71	9.53	4.13
138/22	19.23	2.84	11.83	4.36
138/32	20.27	3.50	10.41	4.36
138/43	22.20	1.13	10.93	5.16
138/78	19.87	3.50	13.44	5.76
126/1	19.30	3.17	10.58	4.27
126/5	19.67	3.46	11.51	4.49
CD (P<0.05)	0.25	0.49	0.29	

Chromosome number analysis of seedling progenies

Chromosome analysis was completed in 96 seedling progenies. All of them showed deviation from the normally reported chromosome number of 2n=63. Most frequently observed chromosome number was 2n=84. Variations observed were 2n=65, 2n=78, 2n=85, 2n=86, 2n=87, 2n=88 etc.

Mining of DNA markers and genes from Expressed Sequence Tags

A short and easy protocol suitable for isolation of RNA was standardized. The protocol utilizes 2% SDS, 2% PVP, 100 mM Tris Cl and 25





mM EDTA in the extraction buffer followed by acid phenol extraction in presence of 5M sodium acetate. This protocol can be commonly used for RNA extraction from leaf and rhizomes and gives atleast 5 times more yield than available protocols.

Development of SSR enriched libraries and microsatellites

The DNA fragments were enriched for SSR regions by hybridizing with biotinylated microsatellite oligos probe mix [(ACT)12, (AAAC)6, (ACCT)6],(ATC)8,[(AAT)8, (GCC)8,(ATT)8, (ACC)8, (GATA)6] and captured using streptavidin coated dynabeads. Eluted fragments were again enriched by PCR and ligated to pTZ57R/T (Thermo scientific) and TOPO 2.1 (Invitrogen) cloning vectors and transformed into both TOP 10 competent cells and *E. coli* DH5α cells (Invitrogen) as described by the manufacturer. A total of 500 colonies were obtained. Out of this plasmid DNA was isolated from 240 colonies and confirmed by colony PCR (Fig 4.1). After restriction digestion of the plasmid DNA the positive samples were sent for sequencing (Scigenome, Cochin). The sequences were analyzed and primers were designed for the microsatellite regions. Newly designed primers were validated in selected accessions and seven polymorphic markers were validated (Fig 4.2) and added to the microsatellite marker repository.

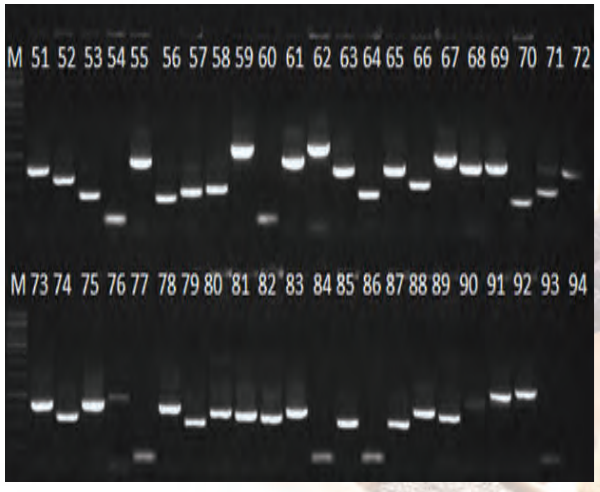


Fig 4.1 Colony PCR confirming presence of SSR repeats; M- 1 Kb DNA ladder.

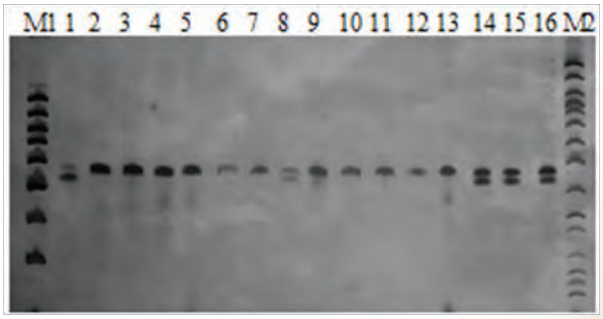


Fig 4.2 Profiling of different *Curcuma* accessions in 15% denaturing PAGE using primer CuMisat 41 (F: ATGTCAGATGTGGATGTTACGC, R: TTCTACTGTCCTGTCTCCGGTT); lanes 1 Alleppey supreme, 2. Amalapuram, 3. Jobedi, 4. Kasturi, 5. Ayur, 6. Jorhat, 7. Sudarshana, 8. Kedaram, 9. Suguna, 10. Arunachal, 11. Manipur, 12. Dilburgh, 13. Lakadong, 14. Prabha, 15. Pratibha 16. Suvarna, M1-100bp DNA ladder, M2-50bp ladder

Source-sink relationship

Samples of varieties IISR Prathibha, IISR Alleppey Supreme and Suguna were taken from 50 DAP. Plant height, number of leaves, leaf area and dry weight (DW) were recorded in these varieties at monthly intervals. All the varieties were on par with regard to leaf area and dry weight at all sampling dates. Starch content increased steadily from 50 to 110 DAP and then there was sudden increase in all the varieties. All the varieties accumulated more biomass in shoot compared to rhizomes up to 110 DAP (Table 4.5). After 110 DAP, there was sudden rise in partitioning of biomass to rhizomes compared to shoot. At 150 DAP, rhizomes accumulated >75 % biomass.

Table 4.5 Partitioning in different varieties

Days after planting	Total DW* plant^{-1} (g)	Shoot (%)	Rhizome (%)	Root (%)
IISR Prathibha				
50	5.9	84.4	8.4	7.0
80	21.0	73.4	20.8	5.8
110	34.2	59.2	35.4	5.4
150	58.0	17.0	79.0	4.0
IISR Alleppey Supreme				
50	6.2	85.4	7.4	7.2
80	18.9	75.2	18.8	6.0
110	28.1	62.3	32.1	5.6
150	50.0	18.0	77.0	5.0
Suguna				
50	5.1	82.2	9.8	8.0
80	20.2	70.4	23.6	6.0
110	31.6	60.8	37.8	5.4
150	52.5	18.2	78.4	3.4

*dry weight

Endogenous IAA and zeatin riboside levels were quantified in both leaves and rhizomes. Both leaves and rhizomes had similar levels of hormones (Table 4.6). IISR Prathibha recorded slightly higher concentration in leaves while Suguna showed higher concentration in rhizomes. Total hormone concentration was more in IISR Alleppey Supreme compared to other varieties. All the varieties showed maximum levels at 110 DAP. Photosynthetic rate, stomatal conductance and transpiration rate were recorded at 100 and 130 DAP. Photosynthetic values ranged from 5.0-7.8µmoles. All varieties showed higher photosynthetic rate at 130 DAP compared to 100 DAP. Suguna showed lowest values both at 100 (5.0µmoles) and 130 DAP (6.5µmoles) .

Table 4.6 Starch and plant hormones in different varieties

Days after planting	Rhizome starch (%)	IAA (µ moles)	Zeatin riboside (µ moles)
IISR Prathibha			
50 80 110 150	4.3 8.9 14.7 36.4	14.6 26.4 52.1	9.8 20.7 40.4
IISR Alleppey Supreme			
50 80 110 150	4.0 8.1 13.9 37.5	12.6 29.4 47.6	10.6 18.9 44.0
Suguna			
50 80 110 150	5.0 8.6 15.3 35.7	15.7 27.8 53.2	11.8 23.0 39.5

Crop Production

Organic farming

An organic package consisting of 20t FYM + 2t Neem cake + 1t Ash + 4t Vermicompost ha^{-1}, *Azospirillum* and P solubilising bacteria (20g bed^{-1}) for nutrient supplement, GRB35 as seed treatment and application of Panchagavya as drench and spray at 45 and 90 DAP has been standardized. Drench of GRB35 or 57 and GEB17 are helpful in keeping the disease incidence to <10-15%. Spray of BM (1%) to contain foliar diseases and neem oil (5mL L^{-1} of water) in combination with the cultural control is recommended for shoot borer. Results showed that organic management system yielded on par with integrated systems and curcumin content was found to be higher under organic systems.

Micronutrients on yield and quality

Based on the field studies on effect of Zn and B on the quality of variety. IISR Prathibha for 3 years, soil application of Zn up to 10kg ha^{-1} or foliar spraying of $ZnSO_4$ (0.25%) and Borax (0.2%) twice (60 and 90 DAP) is recommended for high yield and quality in Zn and B deficient soil (Table 4.7).

Table 4.7 Effect of Zn & B on curcumin content (%) (pooled mean of 2008-11)

Treatment	Zn			Treatment	B		
	-P	+P	Mean		- Lime	+ Lime	Mean
Zn-0	4.89	5.00	4.9	B-0	4.5	4.9	4.7
Zn-5	4.62	5.11	4.9	B-1	4.3	5.0	4.6
Zn-10	4.75	5.25*	5.0	B-2	4.2	4.9	4.6
Zn-15	4.68	5.10	4.9	B-3	4.3	5.2	4.8
FS-1	4.72	5.10	4.9	FS-1	4.8*	5.3*	5.1*
FS-2	5.29*	5.46*	5.4*	FS-2	4.6*	5.1	4.8
CD (P<0.05)	P x Zn - *		0.26		L x B - *		0.30





Crop Protection

Biochemical characterization of mo derately resistant and susceptible accessions to shoot borer

Total fibre and lignin contents in mature shoots and total carbohydrates, lignin and proteins in mature leaves were estimated in both shoot borer resistant and susceptible accessions. Lignin, total proteins, phenols, carbohydrates and fibre contents in immature leaf tissues and shoots were determined in susceptible and moderately resistant accessions. Epicuticular wax was estimated only in immature leaf tissues (Table 4.8).

Table 4.8 Biochemical characterization of resistant and susceptible accessions

Character	Mature				Tender			
	Leaf		Shoot		Leaf		Shoot	
	MR	S	MR	S	MR	S	MR	S
Phenol (mg 100mg^{-1})	-	-	-	-	0.8–1.5	1.1–1.8	0.12–0.14	0.11– 0.15
Carbohydrate (mg 100mg^{-1})	7.5–19.7	8.8–12.7	-	-	-	-	-	-
Protein (mg 100mg^{-1})	1.1–7.7	2.8–11.1	-	-	-	-	-	-
Lignin (%)	26.4–48.7	23.9–39.1	10.6– 35.6	28.1–32.8	36.8–42.0	36.5–42.0	43.1–44.3	25.7–37.0
Fibre (%)	-	-	22.6–32.7	21.2–31.8	18.7–22.0	15.0–22.0	42.7–44.4	24.3–30.3
Epicuticular wax (mg 75 cm^{-2})	-	-	-	-	44.7–45.3	45.0–46.0	-	-

MR=Moderately Resistant; S=Susceptible





Evaluation of EPNs

The infectivity of 4 promising EPNs *viz., Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius gingeri* and *Oscheius* sp. (IISR-EPN 08) was tested against shoot borer larva in the greenhouse. The treatments included application of the EPNs @ 25000IJs pot^{-1} at 21 days interval during August to October, spraying of Malathion 0.1% (present recommendation) and control. Shoot damage was recorded at the end of crop season. Among the test EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* treated plants showed minimum shoot damage (26.1% and 26.6%, respectively) which was significantly superior over control (44.4%) and was on par with insecticide application.

Mass production of promising EPNs

In vitro multiplication of 4 promising EPNs *viz., Heterorhabditids* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *O. gingeri* and *Oscheius* sp. (IISR-EPN 08) in 3 modified Wouts medium (MWM) was tested. The ingredients of the modified media were mixed and coated on to foam pieces. The flasks were filled with foam media mixture and sterilized. After cooling at room temperature, 1000IJs were introduced into each flask and sealed. The flasks were incubated at 30^{o}C for 25 days. Colonies of nematodes started appearing on the walls of the flasks 2 weeks after inoculation. The IJs were harvested through sieving and decantation method and EPNs counted with the help of Syracuse counting dish. Among the test media, all test EPNs were produced in MWM-I. However, maximum numbers of *O. gingeri* were produced in MWM-II. No multiplication of EPNs was observed in MWM-III.

5. VANILLA

Maintenance of germplasm

Ninety three germplasm collections and 300 seedling progenies/interspecific hybrids were maintained in nursery in pots/bags

Screening of interspecific hybrids for disease resistance

Ten plants each of interspecific hybrids involving *V. planifolia* x *Vanilla* sp. (A&N) and *V. planifolia x V. tahitensis* and 15 plants of *V. planifolia x V. aphylla* were inoculated with *F. oxysporum*. After the first round of inoculation most of the plants took infection. Those that survived infection were given second round of inoculation.

Maintenance of *in vitro* cultures of vanilla

In vitro cultures of 360 seedlings from 7 collections and 70 inter-specific hybrids of 5 combinations were maintained. Fifty interspecific hybrids between *V. planifolia* and *V. aphylla* and 50 interspecific hybrids between *V. planifolia* and *V. tahitensis* were established *ex vitro*.

Morphological and cytological analysis of interspecific hybrids

Morphological characters namely leaf length, leaf breadth and inter node length were recorded from 10 interspecific hybrids involving *V. planifolia* and *V. tahitensis*. Variation in these characters among the interspecific hybrids was evident. Chromosome number analysis of 2 interspecific hybrids showed 2n=30 in one and 2n=32 in other. Interspecific hybrids between *Vanilla* sp. (A&N Islands-White) with wide leaves and *V. aphylla* without leaves showed leaf less nature of hybrid progenies in the juvenile stage (Table 5.1). However, they developed leaves after 1-2 years of growth in the nursery. These leaves also showed variation in size and more similarity in shape to *V. pilifera*. In a few progenies, the flowers were smaller than that of *Vanilla* sp. (A&N) and slightly bigger than that of *V. aphylla*. In general the flower architecture was similar to that of *V. aphylla* but, the frill of labellum was similar to that of *Vanilla* sp. (A&N). These hybrids showed 100% pollen sterility and failed to set fruits on self pollination. However, pollination with *V. aphylla* pollen in these hybrids resulted in fruit set.

Hybrid seedlings between *V. planifolia* and *V. aphylla* showed presence of well developed leaves right from the *in vitro* seedling stage and no hybrids without leaves were observed. These hybrids are at their second year of *ex vitro* establishment and have normal growth.

Table 5.1 Morphological variation (Mean±SE) among 5 interspecific hybrids from a cross between *Vanilla* sp (A&N) x *V. aphylla.*

Identity	Leaf length (cm)	Leaf breadth (cm)	Internode length (cm)	Girth (cm)
AN x Aphy-1	12.75±0.44 B	2.04±0.08 B	10.53±0.42 AB	2.66±0.04 C
AN x Aphy-2	9.46±0.23 CD	1.61±0.07 C	10.80±0.38 AB	2.78±0.05 BC
AN x Aphy-3	12.62±0.57 B	2.28±0.10 B	11.32±0.33 A	2.84±0.05 BC
AN x Aphy-4	10.43±0.46 C	1.72±0.07 C	11.56±0.34 A	2.76±0.11 BC
AN x Aphy-6	8.77±0.29 D	1.57±0.07 C	10.00±0.20 B	2.90±0.07 B
Vanilla sp.	17.79±0.59 A	5.0±0.15A	9.92±0.43 B	3.36±0.10 A





6. TREE SPICES





NUTMEG

Selection of promising clones

Surveys were conducted in farmers' fields in adjoining areas of Pala and Thodupuzha and 6 nutmeg accessions were collected. They are *Cheripurathu* (entire mace), *Madukkakuzhi* (bisexual type, producing large number of clustered fruits), 2 yellow mace types, *Kinattukara* and *Kochukudi* (both high yielding types). In the trial on evaluation of lines for high yield, significant differences were observed among the lines for all the 4 characters and A9/185 was found to be the best with regard to plant height (7.0m), canopy (5.25m), girth (70.5cm) and number of branches per plant (48).

Pruning studies

Shoot tip pruning (20cm) was done during August and flowering was observed on new shoots which emerged on pruning. However, pruning did not influence the total number of flowers produced per shoot.

GARCINIA

Total fat content in garcinia seed butter was 40% indicating it as an important source of fatty acids. It was solid at room temperature and almost as hard as cocoa butter (Table 6.1). Melting point was high and free fatty acid content was very less.

Molecular study

Sequencing of ITS region of 9 species of garcinia (4 from Western Ghats and 5 from NE Himalayan foot hills) was done and deposited in NCBI data base. The ID numbers ranges from LOCUS no JX472233-JX472241. Evolutionary relationship tree was prepared with the sequence data. In each cluster one number from Western Ghat and another number from Himalayan species with almost similar size color and shape joined.

Table 6.1 Biochemical composition of the seed butter of garcinia Species

Parameters	*G.gummi-gutta*	*G.indica*	*G.tinctoria*	*G.mango-stana*
Chemical Properties				
Acid Value (mg NaOH g^{-1} of oil)	3.7	4.9	4.8	4.5
Saponification number (mg KOH g^{-1} of oil)	187.9	200.2	190.3	140.5
Iodine value	50.2	39.4	37.4	51.8
Free fatty acids (%)	1.42	5.64	2.82	2.21
Fatty acid composition (%)				
Palmitic acid	6.31	3.25	3.05	47.20
Stearic acid	30.61	49.33	44.53	2.31
Elaidic acid	9.54	3.00	1.51	-
Oleic acid	26.23	34.42	35.33	34.02
Linoleic acid	11.38	5.25	4.82	1.32
Arachidic acid	5.41	1.20	1.00	8.04
Eicosenoic acid	ND	2.25	1.01	0.51
Other fatty acids	10.52	2.30	8.75	6.61
Sterols	0.54	1.02	0.96	0.10
Vit. E (mg $100g^{-1}$)	14.31	20.01	-	-
Total saturated FA	48.64	52.78	48.58	57.55
Mono unsaturated FA	35.77	39.67	37.85	34.53
Poly unsaturated FA	11.38	5.25	4.82	1.32
Other properties				
Vit. E (mg $100g^{-1}$)	14.31	20.01	9.0	12.0
Total saturated FA	48.64	52.78	48.58	57.55
Mono unsaturated FA	35.77	39.67	37.85	34.53
Poly unsaturated FA	11.38	5.25	4.82	1.32

7. POST HARVEST TECHNOLOGY, VALUE ADDITION AND HIGH VALUE COMPOUNDS





Cryogenic grinding of spices

Black pepper, turmeric and cinnamon were powdered using 3 different means such as ambient ground, freshly ground and cryo-ground. The powders obtained from CIPHET, Ludhiana were subjected to quality evaluation and antioxidant studies. The total phenol, antioxidant activity on the basis of DPPH assay, Phosphomolybdenum method and FRP method using alcohol, water and petroleum ether extracts of turmeric variety Prathiba did not show any variation (Table 7.1 & 7.2). This shows that grinding method does not influence the antioxidant property.

Table 7.1 Chemical quality of cryo ground and ambient ground samples of turmeric

Samples	Moisture (%)	Essential oil (%)	Oleoresin (%)	Curcumin (%)
Ambient ground	9.4	2.0	10.7	3.4
Cryo ground	11.2	2.4	11.1	3.5
Freshly powdered	9.4	2.4	10.9	3.7

Table 7.2 Total phenol and antioxidant property of variety IISR Prathibha ground using different methods

Sample	Phenol			DPPH Assay			Phosphomolybdenum			FRP method		
	A	W	P	A	W	P	A	W	P	A	W	P
Ambient ground	1.66	4.62	0.48	87.85	36.54	14.48	13.40	44.24	10.48	191.46	52.50	33.72
Cryo ground	1.56	4.68	0.49	87.32	35.79	14.59	13.44	44.94	10.51	192.52	52.54	34.10

A- Alcohol W- Water P- Petroleum ether

The cryo ground black pepper Panniyur-1 yield high oleoresin compared to other methods. Freshly ground sample gave high oil and comparable oleoresin. Piperine content did not show any variation. GC MS analysis of essential oil from black pepper Panniyur-1 indicated that major sesquiterpenes like β–Caryophyllene gave better yield in cryoground sample. Except for β–Phellandrene, no major difference was observed in oil constituents

Spice extracts for anticancer effect

Telomerase activity is negative in human normal somatic cells but detected in more than 85% of tumor cells. Therefore, targeting telomerase enzyme is an attractive proposition in the treatment of cancer. Thus, in this study, *in silico* methods were used to identify inhibitors of telomerase from major spice phytochemicals with ADMET satisfaction, used as ligands, *viz.*, curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, piperine, cinnamaldehyde, 1,8-cineol, myristicin, beta-caryophyllene and alpha-pinene. The targets sourced from the NCBI database were:

- 3KYL, the catalytic subunit of telomerase bound to its RNA template and telomeric DNA
- 2R4G, RNA-binding domain of telomerase and
- 2B2A, the essential N-terminal domain of telomerase reverse transcriptase.

The ligands and the targets were optimized using ArgusLab 4.0.1. The phytochemicals were docked to the active-sites of the above tar-





gets to assess binding affinity and consequently their inhibitory activity using Molegro Virtual Docker Version 5.5.0. The post-docking analyses revealed that the turmeric bioactive principle - curcuminoids I, II and III (curcumin, demethoxycurcumin and bisdemethoxycurcumin respectively) and tetrahydrocurcumin showed consistently superior docking ability to the telomerase targets, with high scoring functions that bind to the selected targets with strong binding efficiency and hydrogen bond interactions. Piperine and myristicin, though less efficient, had greater interaction than the remaining ligands. These phytochemicals can be potentially developed as lead drug molecules against cancer, by targeting telomerase.

Curing of turmeric in an improved boiler

Studies on curing and drying of turmeric were conducted at Central Institute of Fisheries Technology, (CIFT), Kochi. Experiments on cooking of turmeric at three varying steam pressures (0, 0.5 and 1kg cm^{-2}) corresponding to steam temperatures of 100, 112 and 121°C for four different cooking durations (5, 10, 15 and 20 min) was performed in a pilot-scale rotary retorting system (Model 24, John Fraser and Sons Ltd., Newcastle-upon-Tyne, UK) to determine the time required for complete drying and to evaluate the quality of the dry turmeric obtained. The cooked samples were dried in CIFT-CRYER-SDL 250 model solar drier from 9.30am to 4.30pm. The samples were left inside the drier with fans on overnight and the drying process continued till the samples were uniformly dry. Studies indicated that irrespective of the treatment combinations, the drying time required for complete drying was 7 days.

Studies on bulk curing and drying of turmeric were also conducted at CIFT, Cochin. About 75kg was cured in a horizontal retort at 1kg cm^{-2} pressure for 15 min and dried in CIFT-CRYER-SDL 250 model solar drier from 9.30am to 4.30pm. The study indicated that drying was completed in 7 days.

Experiments on curing of turmeric (variety IISR Prathibha) was conducted in improved turmeric boiler (TNAU model) and conventional water boiling methods for various curing durations. Cured turmeric was sun dried in cemented yard to reduce the moisture content of fresh rhizomes from 80.50% to less than 10%. Turmeric cured by traditional water boiling method for 45, 60 and 90 min and those cured in improved steam boiler for 30, 45, 60 and 90 min took 10 days for complete drying. But in case of rhizomes dipped in boiling water for 10 min and then dried, the drying time increased to 13 days. When the rhizomes were sliced and dried, the drying time reduced to 8 days. Quality analysis of the dry turmeric samples indicated a marginal reduction in curcumin content from 4.66-4.44% when the curing time increased from 30 min to 90 min in case of improved curing method and from 4.95-4.62% when the curing time increased from 30 min to 90 min in case of water boiling method.

Production of food extrudates

The cassava flour was mixed with cardamom power in the ratio of 96:4 and conditioned at 4°C for 15 days. The blend was extruded in a single screw stand alone Brabender extruder at a die temperature ranging from 170-190°C at a screw speed ranging from 70-90rpm and by using Response Surface Methodology, the machine parameters of the extruder were optimized to get optimum quality of the extrudates based on the physical, functional, textural, biochemical and sensory properties of the extrudates from the flour blend of cassava and cardamom.

From the regression models obtained it was found that the expansion ratio would be at a maximum of 3.85 at a die temperature of 170.1°C and at a screw speed of 79.1rpm, the bulk density would be at a minimum of 0.19g mm^{-3} at a die temperature of 172°C and at a screw speed of 76rpm, the porosity would be at the maximum of 62.83% and at a die temperature of 170.2°C at a screw speed of 71.4rpm, the water absorption index would be at the minimum of 5.2 at a die temperature of 173.6°C and at a screw speed of 70.7 rpm, the water solubility index would be at the

maximum of 2% at a die temperature of 170.1°C and at a screw speed of 82.3rpm, the oil absorption index would be at a minimum of 2.3 at a die temperature of 172.4°C and at a screw speed of 73.6rpm, the hardness would be at a minimum of 38N at a die temperature of 170.1°C and at a screw speed of 79.3rpm, the toughness would be at a minimum of 34 Nm and at a die temperature of 170.3°C at a screw speed of 79.67rpm, the crispness would be at a maximum of 31.7 numbers of peaks and at a die temperature of 179.9°C at a screw speed of 89.9rpm, the retention of cineol would be a maximum of 23.82mg $100g^{-1}$ of sample at a die temperature of 177.2°C and at a screw speed of 89.0rpm, retention of terpinyl acetate would be at a maximum of 34.74mg $100g^{-1}$ of sample at a die temperature of 183.9°C and at a screw speed of 89.1rpm and the overall acceptability scores would be at a maximum of 6.75 at a die temperature of 170.6°C and at a screw speed of 76.7rpm.

Chemoprofiling of turmeric and ginger varieties

Essential oil profile of rhizhomes of seven varieties of turmeric, *viz.*, Sona, Sobha, Varna, Rajendra Sonia, Narendra Haldi, Sugandham and Co-1 was determined by GC-MS and these contained turmerone (5.6-25.8%), ar-turmerone (3.5-20.8%) and curlone (5.4-15.6%) as the chief components. ar-turmerone ranged from 14 -20.8 % in all tested varieties except Sobha with 3.5%. Turmerone level was 20-25% in Rajendra Sonia, Sugandham, Narendra Haldi, and Co-1; 5-7% in Sobha and Sona and 13% in Varna. Varna, Rajendra Sonia, Sobha and Sona contained 5.0-6.8% curlone and Sugandham, Narendra Haldi and Co- I with slightly higher level, (12.5-15.6%). There was not much variation in the contents of the minor and trace components (Table 7.3).

In ginger, volatile oil profile of rhizomes of seven varieties *viz.*, Varada, Mahima, Rejatha, Suprabha, Surabhi, Himgiri and Rio de Janeiro was determined. The major constituents were zingiberene (16-23%), farnesene (9-12%), β-sesquiphellandrene (11-12%) and ar-curcumene (8.9-10.3%). Zingiberene varied from 20-23% in all varieties except Rio-de Janeiro (16.4%); ar-curcumene was comparatively low in Varada (5.7%) whereas it ranged from 8.9-10.3% in others (Table 7.4).

Table 7.3 Essential oil profile of turmeric varieties

	Composition (%)						
Constituents	Sugandham	Narendra Haldi	Co -1	Varna	Rajendra Sonia	Sobha	Sona
α-pinene	0.22	0.03	0.13	0.07	0.12	0.26	0.08
sabinene	0.00	0.04	0.00	0.00	0.03	0.02	0.00
Δ3-carene	3.28	0.15	0.33	0.08	0.28	0.02	0.02
α-phellandrene	3.59	0.00	1.11	0.32	0.85	0.78	0.00
limonene	0.61	0.37	0.36	0.19	0.36	0.22	0.18
1,8-cineol	4.78	3.91	1.73	4.30	2.04	6.61	5.76
γ-terpenene	0.41	0.04	0.10	0.04	0.08	0.04	0.00
terpinolene	0.89	5.43	12.28	4.94	9.95	0.53	1.65
caryophyllene	0.20	1.57	1.08	0.40	1.96	0.20	0.27
a-terpineol	0.49	0.00	0.13	0.42	0.55	0.91	0.00
zingiberene	0.98	1.21	2.86	2.01	1.58	2.46	1.99
β-bisabolene	0.18	0.46	0.60	1.20	0.42	1.92	1.45
ar-curcumeme	0.65	1.28	1.05	1.52	1.40	1.38	0.00
turmerone	25.76	20.35	22.57	13.25	20.91	5.60	7.11
curlone	15.64	14.12	12.45	4.97	5.35	5.87	6.82
Ar-tumerone	16.17	20.84	15.96	18.62	18.21	3.45	13.98

Table 7.4 Essential oil profile of ginger varieties

	Composition (%)						
Constituents	Varada	Suprabha	Himgiri	Rejatha	Mahima	Rio de Janeiro	Surabhi
α-pinene	0.55	0.58	0.54	0.74	0.59	0.54	0.71
camphene	1.84	1.80	1.61	2.47	1.89	1.70	2.38
sabinene	0.04	0.00	0.03	0.04	0.04	0.03	0.03
β-pinene	0.06	0.03	0.06	0.07	0.08	0.06	0.07
β-myrcene	0.71	0.06	0.71	0.58	0.80	0.62	0.62
α-phellandrene	0.15	0.15	0.00	0.16	0.17	0.13	0.15
β-phellandrene	3.17	2.82	2.27	3.71	3.83	2.74	3.09
terpinolene	0.15	0.14	0.00	0.16	0.15	0.12	0.13
linalool	1.45	1.76	1.06	1.84	1.45	1.50	1.25
α-terpeniol	0.40	0.00	0.35	0.43	0.58	0.36	0.33
β-citronellol	0.23	0.38	0.28	0.30	0.00	0.29	0.00
cyclosativene	0.26	0.27	0.27	0.26	0.26	0.27	0.25
α-copaene	0.46	0.48	0.48	0.44	0.50	0.48	0.45
β-elememe	0.86	0.92	0.88	0.83	0.89	0.93	0.86
ar-curcumene	5.65	9.21	9.93	8.86	9.43	10.34	9.21
zingiberene	23.04	23.23	20.07	22.4	20.84	16.37	23.15
farnesene	11.86	9.37	11.63	11.68	11.83	9.70	10.47
β-bisabolene	1.94	2.23	1.99	2.06	2.12	2.21	2.63
β-sesquiphell-andrene	11.96	11.76	11.72	11.60	11.12	11.19	12.31





8. EXTENSION AND IMPACT ASSESSMENT





Soil based nutrient management plans for agro-ecosystems of Kerala

The project envisages analyses of 17,069 soil samples of 87 Panchayats of Calicut district, analyses for pH, OC, major-, secondary- and micro-nutrients, uploading the data into www.keralasoilfertility.net. Subsequently nutrient advisory cards will be generated followed by identification of production potential of each AEZ, development of nutrient management plans for our AEZ, and development of GIS maps for integration with the NREDB database generated as part of ISRO funded project. As on date, 10110 soil samples representing 47 Panchayats have been analyzed for pH, EC, major- and secondary- and micro-nutrients. Results of 9220 soil samples representing 43 Panchayats have been uploaded into www.keralasoilfertility.net.

The salient findings are as under:

- Most of the soils (93%) were acidic, with 51% of the soils being strongly acidic (pH < 5.0).
- Majority of the soils (71%) were medium to very high in organic C (0.8-5.0%), while 29% of the soil samples were low to very low (<0.7%) in organic C.
- The available P levels were high (25-100kg ha^{-1}) in 64% of the soils analyzed, while 19% of the soils were deficient (5-10kg ha^{-1})
- With regard to available K, 51% of the samples were medium (116-275kg ha^{-1}), 29% of the samples high to very high (400-1000kg ha^{-1}) and 16% of the samples were low to very low (75-115kg ha^{-1}).
- In case of secondary nutrients, 60% of the samples were adequate (>300kg ha^{-1}) in exchangeable Ca; conversely, 81% of the samples were low to very low (60-120kg ha^{-1}) in exchangeable Mg.
- In case of micronutrients, 91% of the samples were adequate in Zn, 96% were adequate in Cu, 49% adequate in B, while 33% of the samples were low in S.

Technology mission for pepper in Wayanad

About 750 soil samples from Thirunelli panchayat of Wayanad district were analysed for major, secondary and micro nutrients and results with site specific recommendations were passed on to the farmers. Study on the analysis of *R. similis* population associated with yellowing of black pepper was studied in soil samples collected from rhizosphere of black pepper from 50 fields of Thirunelli, Poothadi, Mullankolli and Pulpalli villages of Wayanad. *R. similis* was more frequent in Mullankolli (AF=31.37%) followed by Pulpalli (AF=29.41%), whereas, less frequent in Thirunelli (AF=17.64%). However, *R. similis* was more abundant and prominent in Pulpally (D=29.94; PV=1.62), followed by Poothadi (D=23.39; PV=1.08) whereas, least abundant and prominent in Thirunelly (D=10.27; PV=0.43) (Fig 8.1).

Pamphlets were prepared in Malayalam on composting, use of pesticides, Biocontrol of pest and diseases and distributed to farmers. Twenty five farmer's plots were selected in three panchayats and FLDs on varieties and technologies to rejuvenate yellowing of black pepper is initiated and the inputs like planting material, lime, organic manures, neem cake, micro nutrient mixtures and bio agents were supplied (Fig 8.2).

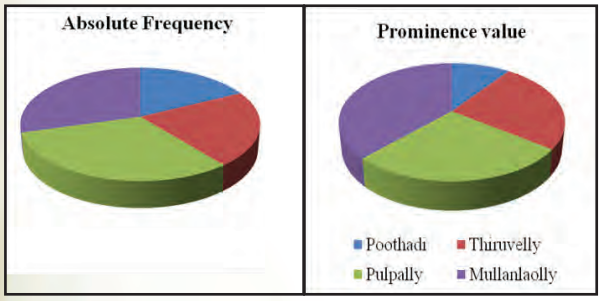


Fig 8.1 Population analysis of nematodes in Wayanad district

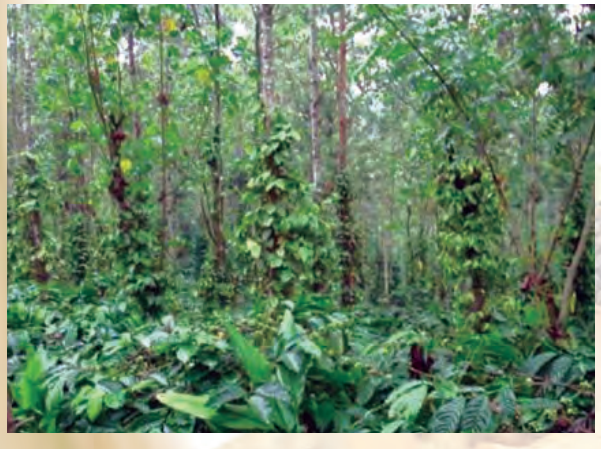


Fig 8.2 FLDs on management technology of black pepper at farmer's field in Wayanad

Success stories of IISR Prathiba turmeric from farmer's plot

Feed back from farmers across different states of India attested the acceptability of IISR Prathibha turmeric. In a farm at Vellamunda, Wayanad, IISR Prathiba is grown at 16 acres of land (Bucca farms) (Fig 8.3). Similarly in Andhra, many farmers now opt for IISR Prathiba over the traditional varieties of turmeric (Fig 8.4 a&b). The yield expected is 35-40t ha^{-1}. ICAR (www.icar.org.in) displayed the success story in it's home page.

Address of farmer/size of plot	Fresh yield (t $acre^{-1}$)
Mr. Chandra Sekhar Azad, Guntur (2.5 acres)	16.0
Mr. Ramesh Reddy, Guntur (2 acres)	17.0
Mr. Sivarama Krishna, Guntur (2 acres)	15.0
Mr. Krishna, Guntur (0.5 acre)	16.0
Mr. G. Venkatappaiah, Guntur (3 acres)	15.0
Mr. Muhammed Bustani, Wayanad (17 acres)	16.5

Fig 8.3 Mr. Muhammed Busthani in his IISR Prathibha field

Fig 8.4a IISR Prathibha field of Mr. Venkatappaiah, Guntur

Fig 8.4b IISR Prathibha field of Mr. Chandra Sekhar Azad, Guntur





9. ALL INDIA COORDINATED RESEARCH PROJECT ON SPICES





The All India Coordinated Research Project on Spices (AICRPS) is vested with the mandate to conduct and coordinate research in 12 spice crops namely, black pepper, cardamom, cinnamon, clove, coriander, cumin, fennel, fenugreek, ginger, large cardamom, nutmeg and turmeric, with its headquarter at Indian Institute of Spices Research, Calicut. AICRPS has 34 centers which include 19 regular, 8 co-opting and 7 voluntary centres located in 21 states of India under 21 State/Central Agricultural Universities (SAUs)/Research Institutes. The 2012 - 13 budget of AICRPS is Rs. 625 lakhs.

Promising Technologies

Genetic resources

Over 7000 accessions of Spices germplasm is maintained at various AICRPS centres. A few promising lines were short listed in the mandate crops for further evaluation.

At Panniyur the hybrids PRS160 and PRS 161 were the best with maximum green berry yield of 3.2kg vine^{-1} and 3.0kg vine^{-1} respectively. At Kumarganj out of 61 accessions of ginger evaluated, NDG-55 (34t ha^{-1}) was promising with respect to yield.

Crop Improvement

During the year, 9 high yielding high quality varieties of spices were recommended for release. They are ICRI-8 in cardamom, Narendra Haldi-3 in turmeric, PPI (CL)1 in clove, DH 220 and Suguna (LCC-236) in coriander, HF-143 and RF-281 in fennel, Ajmer fenugreek-3 and Rmt-365 in fenugreek and Hisar Ajowain-18 in Ajowan.

In a CVT in cardamom, over the years, genotype CL-722 (289.35kg ha^{-1}) recorded maximum cardamom yield followed by PS-27 (275.58 kg ha^{-1}) than the best check M1 (216.34kg ha^{-1}) at Mudigere.

In a CVT on coriander at Jagudan the entries COR-56 and COR-48 gave higher yield (i.e.,1448 and 1405kg ha^{-1}) than best check GCr-2. At Coimbatore three coriander leafy types *viz.,* CS 1, CS 11 and CS 38 have recorded highest leaf yield of 3250, 4000 and 4275kg ha-1 respectively were identified for off season production.

In CVT on fennel FNL-47 and FNL-56 gave higher yield (ie. 1299, 1285 and 1221kg ha^{-1}) than all the checks.

Crop Production

At Sirsi, initial trials in spices based cropping system black pepper, recommended package of practices gave higher dry berry yield of the black pepper (12kg vine^{-1}) compared to those of organics (0.78kg vine^{-1}).

At Mudigere application of irrigation 9L clump^{-1} day^{-1} along with 100% recommended dose of fertilizer through drip recorded highest cardamom capsule yield.

The results of fertigation experiments in turmeric at Coimbatore revealed application of NPK@150:60:108kg ha^{-1} with urea and potash as straight fertilizers and phosphorus as water soluble fertilizer weekly once recorded a yield of 49.11t ha^{-1}. In a micronutrient trial of turmeric at Pundibari, soil application of boron gave the maximum yield 9.50kg plot^{-1} and 19.15t ha^{-1}, respectively. Soil application of FYM @30t ha^{-1} + vermi compost @20q ha^{-1} + neem oil cake @ 8q ha^{-1} is recommended for higher yield (48.82t ha^{-1}) of organic turmeric at Dholi. However integrated nutrient management involving soil application of inorganic N @150kg ha^{-1} + *Azospirillum* @1.5kg ha^{-1} + FYM @5t ha^{-1} is recommended for higher yield (56.6t ha^{-1}) in turmeric.





In a trial conducted for 3 years to standardize nutrient management for off season production of coriander for leafy purpose at Coimbatore showed that application of 30:40:20 kg NPK ha^{-1} combined with spraying of GA 15 ppm at 20 DAS recorded the highest leaf yield per ha (5870 kg ha^{-1}) with high benefit cost ratio of 2.37. Nutrient supplementation through organic manure using FYM 5.0t ha^{-1} + vermi compost 2.5t ha^{-1} or vermi compost @ 5t ha^{-1} or FYM 2.5t ha^{-1} + vermi compost 3.75t ha^{-1} or FYM 10t ha^{-1} is recommended to increase the seed yield of organic coriander at Coimbatore and Guntur. For the saline soils where there is Zn deficiency (less than 2 ppm) spraying of 0.5% of zinc sulphate (2 sprays at 45 and 60 DAS) is recommended for higher seed yield. To promote micro irrigation and saving water Irrigation with 'Raingun Sprinkler' at either 30 & 60 DAS or 30 & 45 DAS is recommended to improve the productivity of coriander. If water is available for only one irrigation, irrigation with Raingun/Sprinkler at 45 DAS is beneficial and water saving is (58.7%) and 45.3% respectively in Guntur region. Integrated nutrient management with soil application of inorganic N @ 33kg ha^{-1} + Azospirillum @1.5kg ha^{-1} + FYM @ 5t ha^{-1} is recommended for higher yield (1.98t ha^{-1}) of coriander at Dholi region.

The experiment on integrated nutrient management of fenugreek at Dholi, Soil application of inorganic N @13kg ha^{-1} + *Azospirillum* @1.5kg ha^{-1} + FYM @ 5t ha^{-1} is the best and resulted in higher yield of 2.29t ha^{-1}.

Crop Protection

At Sirsi, among the new fungi toxicant molecules @ 0.1 % Fenamidone (10%) + Mancozeb (50 %) (Sectin) alone and Fenamidone (10%) + Mancozeb (50 %) (Sectin) as spraying (@ 2L vine^{-1}) and drenching (3L vine^{-1}) along with bioagent *T. harzianum* (MTCC 5179) 50g with 1.0kg of neem cake as soil application separately during first week of June, 2012 and third week August, 2012 recorded statistically significant reduction in *Phytophthora* foot rot disease of black pepper with regard to leaf infection (6.06% and 4.24%), reduced yellowing of vines (7.88 PDI and 6.67 PDI), least defoliation (8.50 PDI and 6.06 PDI), least death of vines (7.27% and 4.86%) and highest green berry yield (2.93kg vine^{-1} and 3.16 kg vine^{-1}) respectively.

Scale insects of black pepper could be controlled by spraying neem formulation containing 1% Azadirachtin @0.5%, at fortnightly intervals from September onwards (4 applications)

Application of entomopathogenic nematode (EPN) *Heterorhabditis indica* @ 100 Infective Juvenile (IJ) grub was found to be effective in reducing the population of cardamom root grub, when adequate moisture is available in the soil.

Biofumigation using cabbage was found to be the best treatment with highest yield of 6.03 kg plot^{-1} (12.16t ha^{-1})and the lowest bacterial wilt disease incidence (8%). Minimum incidence of soft rot, bacterial wilt, leaf spot and maximum ginger yield was found due to cabbage biofumigation at Solan and Pundibari.

Seed treatment of *Pseudomononas fluorescens* @10g kg^{-1} seed + soil application of *T. harzianum* @2.5kg ha^{-1} and *P. fluorescens* (IISR 6) 10^8 cfu as a spray at 60 DAS is recommended for management of cumin wilt and blight

At Jagudan, application of Thiamethoxam 25WG @ 0.0084% had recorded the least wasp damage (7.44%) and maximum seed yield (2029 kg ha^{-1}) of fennel followed by acetamiprid 20SP@ 0.004% (1906kg ha^{-1}).

Transfer of technology

The AICRP Scientists were also involved in various extension activities like farmer's training, Farm Advisory Service and farm helpline and Agro clinics in collaboration with Department of Agriculture, KVKs, NGOs in various states.

10. BIOINFORMATICS

Development of databases

Details of databases developed during the reporting period are listed below:

- Plant Virus Database: It provides a central source of information about all the plant viruses known to infect plants in India. It includes data on host range, transmission and control, geographical distribution, physical, chemical and genomic properties; taxonomy and relationships; and selected references. It also has genomic sequences of viruses (Fig 10.1a).
- *Phytophthora-Piper* Transcriptome Database: The transcriptome data of *Piper-Phytophthora* interaction is assembled in this database in a searchable format separately for *Phytophthora* and the two *Piper* species *viz. P. nigrum* and *P. colubrinum*.
- Ginger Transcriptome Database: The transcriptomics of *Ralstonia* interaction with *Zingiber officinale* and *Curcuma amada* was compiled in the form of a database (Fig 10.1b).

Besides, sequence information of hybrid assemblies of two isolates of *Phytophthora* infesting black pepper was incorporated to the existing *Phytophthora* Genome Database.

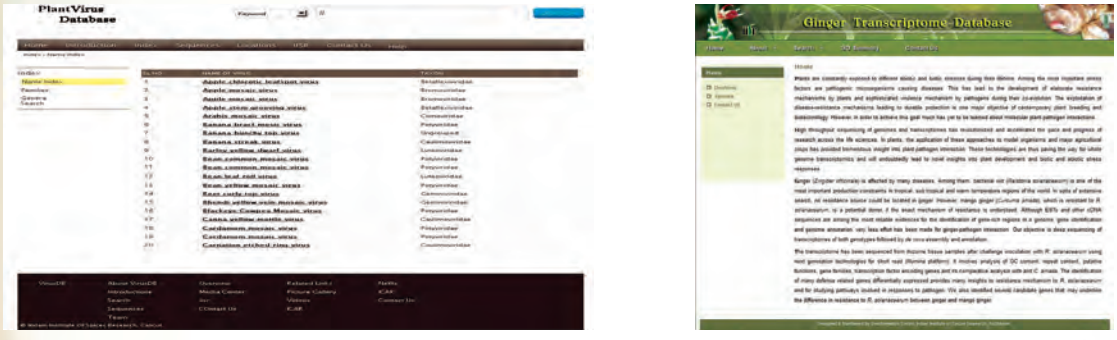


Fig 10.1 a &b Databases on (a) Plant virus; (b) Ginger transcriptome

Phytophthora genome assembly and annotation

Hybrid assemblies of the newly sequenced black pepper isolates of *Phytophthora*, 05-06 and 98-93, were developed and annotated using standard tools. Comparative genomics of these isolates with *P. capsici* was completed and information on SNPs and SSRs was generated. Conserved domain search to identify the protein families present in exonic regions of whole genome sequences of the two different isolates of *Phytophthora* sp. infecting black pepper (05-06 and 98-93) has been carried out along with Blast-2GO analysis.

Species trees were estimated using a concatenation-based multispecies coalescent approach involving Bayesian, maximum parsimony and maximum likelihood methods for a better understanding of their evolutionary history.

Genome mining of endophytic bacteria

Collected information on thousands of chemical compounds involved in various biological processes of 2 endophytic bacteria, *P. putida* and *B. megaterium*, from BioCyc databases (http://biocyc.org/) and from published literature. Predicted compounds having nematicidal activity and having potential fungicidal, oomyceticidal and antiprotozoal activities using PASS Server. *In silico* docking studies are being carried out using a number of target proteins to identify the mode of interaction of the metabolites from *B. megaterium* on *R. similis* and those from *P. putida* against *P. capsici*.





11. AGRICULTURAL KNOWLEDGE MANAGEMENT UNIT

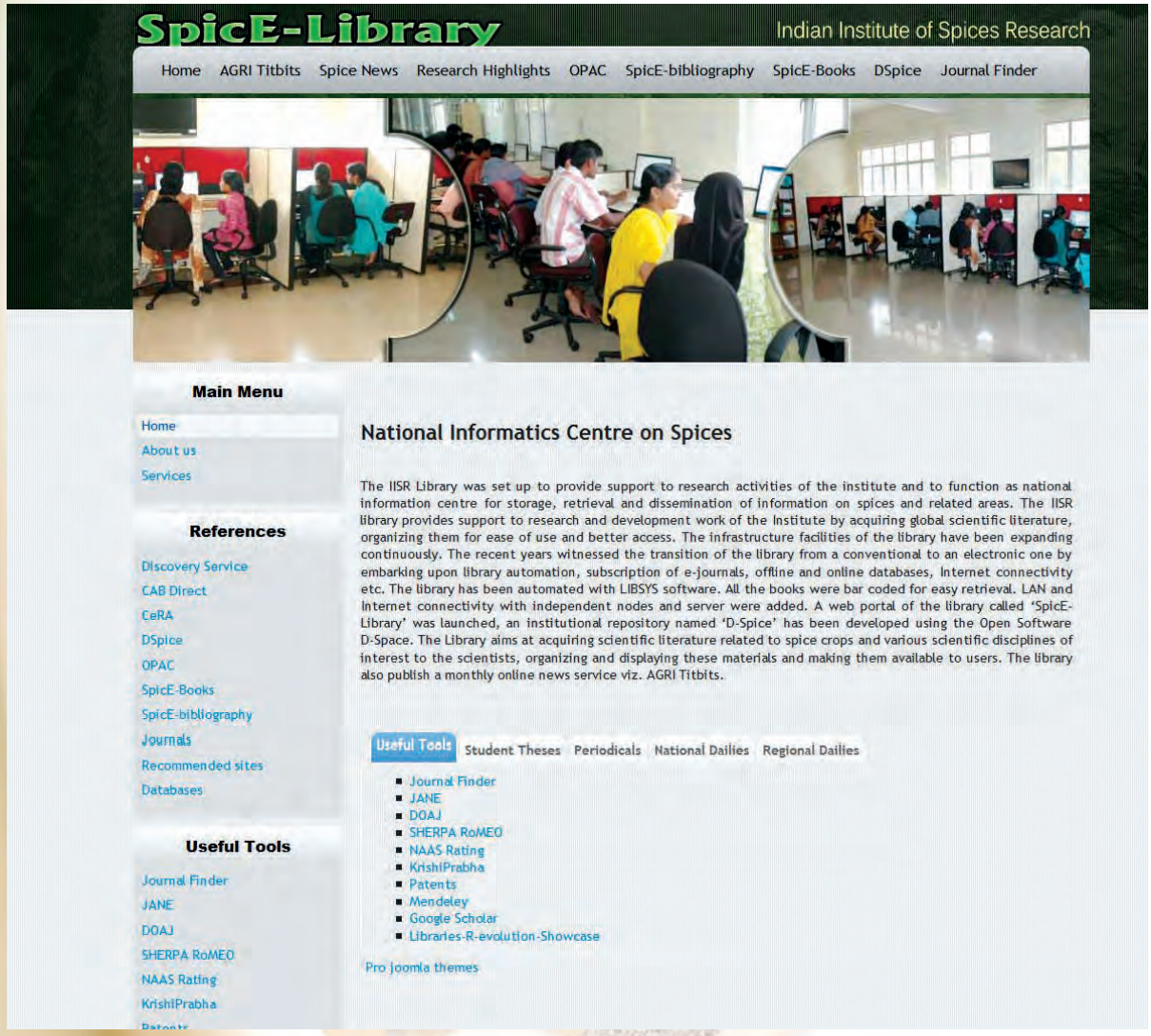
AKMU facilitates the maintenance of the Local Area Network (LAN) of the institute and ensures uninterrupted net connectivity to all divisions/sections. The repair and maintenance of computers, printers and accessories of various divisions/sections is facilitated through AKMU. The Personnel Management Information System Network – II (PERMISnet II) and Project Information & Management System of ICAR (PIMS-ICAR) data is updated from the AKMU. Adding new features in ARISoft and modification and updation of Institute website and intranet portal was also done (Fig 11.1 a & b). Apart from this AKMU assists in statistical analysis of scientific data using SAS and other statistical software.

Fig 11.1a Home page of intranet portal

Fig 11.1b Revamped home page of IISR

12. LIBRARY

The Library website was revamped with links to additional digital resources and databases. The library continued to be a consortium partner of CeRA, the e-journal consortium of ICAR. It subscribed the CAB Direct online, the bibliographic database and 20 foreign journals and 68 Indian Journals. Twelve issues of the online news service 'AGRI titbits' was published. A new multifunctional digital photocopier with scanning and network printing was purchased. Two computers were added to the digital library. A demonstration on Mendely was arranged on 25 October 2012. A demonstration on EBSCO Discovery Service was arranged on 25 October 2012. The scope of digital institutional repository DSpice was widened with more institute publications. The library procured 63 books, 22 technical reports, 7 theses and 6 project reports during the period. Around 156 external users and 2200 internal users made use of the library facilities.







13. AGRICULTURAL TECHNOLOGY INFORMATION CENTRE





Technology inputs

The three technology inputs distributed from the centre include quality planting material of improved varieties of spices, bio control agents and scientific publications including extension literature. During the year 2012-13 planting material for ₹ 228961, publications for ₹ 31405, and bio agents for ₹ 39788/- were sold through ATIC generating a total income of ₹ 3,46,215/-. The proceeds from sale of planting material indicated a 26 % increase compared to previous year and sale of publication an increase of 66 %. The total income generated showed a marginal decrease. The sale of bio agents showed a phenomenal decline over previous year. The trend of component wise income generation through the centre is furnished in Fig 13.1.

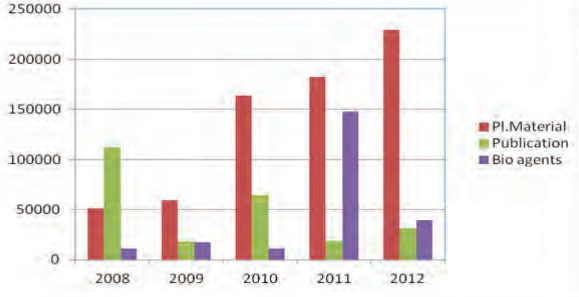


Fig 13.1 Income generation form ATIC

Farmer advisory services

During the year, 1115 farmers availed advisory services through direct visit to ATIC; 452 of them were from Calicut, 362 from other districts of Kerala and 301 from other states. Under ATMA or state sponsorship 12 farmers group availed exposure training by study tour; 5 groups from Karnataka, 5 groups from Kerala and 2 from Maharashtra. 24 students group visited on study tour (18 groups from Kerala, 3 each from Karnataka and Tamil Nadu). Total students visited are 753. The total visit recorded to the centre was 1679. This showed a marginal decrease over the previous year.

From the secondary data recorded on the pattern of information seeking behavior of farmers showed the following trend: Direct visits–1115; Phone calls–358; Letters–115; e mail–124 494 farmers for planting material; 510 for technical advise, 82 for bio agents purchase and 18 for literature and 11 for diagnostic services.

Outreach extension

During the year, the institute participated in 9 exhibitions 3 exhibitions/farmers fares at National level and 2 in state level and 4 in district level.

- Orientation training Programme on Farm Management and Spices Production Technology from 19-21 September 2012 for Technical officer recruits of IISR.
- Training programme on Spices Production Technology for 9 officers from Horticulture Research and Training Centre (HRTC), Jhansi, Department of Horticulture and Food Processing, UP from 11-13 October 2012
- Training programme on Good Agricultural Practices for rural youth sponsored by the Spices Board from 28-30 November-2012 in which 25 trainees from AP and Karnataka attended.
- Course on Production Management in Black pepper for Senior and Assistant Managers and field staff of Ms Harrisons Malayalam Plantations Ltd from 27-28 February 2013 and 19-20 March 2013.
- Under the Horticulture Mission for NE and Himalayan states a Training Workshop on Production management and On farm processing and Post harvest technology of major spices in Arunachal Pradesh was organized in collaboration with Spices Board at Itanagar from 18-20 February 2013 in which 58 farmers from 4 districts of the state and 6 Extension officers from Department of Horticulture participated

Diffusion and Impact Studies

Data collection and analysis on spread of improved varieties of turmeric in Guntur District, Andhra Pradesh and spread of improved varieties of nutmeg and adoption of scientific management practices in Pollachi taluk of Coimbatore district and Udumalpet taluk of Tirupur district were carried out.

The study in Guntur district in Andhra Pradesh revealed that the variety IISR Prathibha has spread in about 250ha as first crop through farmer to farmer lateral spread and exchange of planting material. Even though dominant area is under traditional cultivars like Dugiralla, Kadappa and Tekuurpet, the IISR Prathibha cultivators reported an average yield of 35t ha^{-1}. The reported average yield of local cultivars were fluctuating and mean accounting to 20t ha^{-1}. The perceived advantages of IISR Prathibha are shorter duration, high and stable yield, field tolerance to rhizome rot,(rot is a recurrent problem in the area) and higher dry recovery of 22% compared to 18 % for local. Studies on nutmeg showed that IISR Viswasree has been introduced as a dominant high yielding line as an intercrop in coconut gardens, the buds of established trees widely used for vegetative propagation in Pollachi region of Tamil nadu. Popularisation of the line started since 2000 and the present standing crop is in full bearing stage of around 12 years. The reported average yield under proper shade, canopy management and assured irrigation is 1500 nuts. The various cropping systems identified are Coconut + Nutmeg, Coconut + Nutmeg + Arecanut, Coconut + Arecanut + Banana+ Nutmeg, and Coconut + Cocoa + Nutmeg.

Organising interface for development of media relations

About 10 journalists (Print/TV/Radio) were taken to the turmeric plot of Mr Muhammed Busthani at Wayanad (October 15, 2012). Five journalists participated in the second media visit to IISR Viswashree farmer's field at Karuvarakkundu, Malappuram. Around 10 success stories published in newspapers. One radio programme (Mattoli FM) and TV Feature (Goodness TV).

Organising participatory farmer's mela for showcasing Agricultural Technologies

Dr. S Ayyappan, Secretary, Department of Agricultural Research & Education (DARE) and Director General (ICAR) inaugurated the golden jubilee celebrations of Cardamom Research Centre (CRC) of Indian Institute of Spices Research (IISR) at Appangala in Kodagu district (Karnataka) on 20th December 2012. Shri. K G Boppaiah, Hon'ble Speaker of Karnataka State Assembly inaugurated the Farmer-Scientist interaction held on 21st December. Six progressive farmers from Kodagu who proved that agriculture could be profitable were also honored by the Speaker during the event.

KRISHI DWARA 2012

ICAR Director General also opened Krishi Dwara 2012, a three day showcasing of agricultural technologies. Around 15 agricultural institutes and research organizations exhibited various agriculture related technologies (Fig 13.2).

Fig 13.2 Dr. S. Ayyappan, DG (ICAR) inaugurating Krishi Dwara 2012

Dissemination of innovative technology in print media

More than 20 success stories & 40 news items have been published so far in various malayalam and english newspapers, farm magazines and news portals.

Production of video films and audio capsules

Two video films *viz.*, Bush Pepper to pep up homes and Nutmeg: Money shedding tree were produced in hindi, malayalam and english. Seven audio capsules in Kannada were produced and broadcast through AIR Madikeri.





14. KRISHI VIGYAN KENDRA





Training programmes

KVK has conducted 154 training programmes for practising farmers and farm women, rural youth and extension functionaries in the disciplines of agronomy, horticulture, animal sciences, home science, fisheries, plant protection and allied fields. A total of 6587 trainees were benefited out of the programmes.

FLD Programmes

Fourteen FLD programmes were undertaken during the period as detailed below.

- Demonstration of foliar application of banana micronutrient mixture in nendran banana for higher yield
- Demonstration on use of bio control agents in paddy
- Demonstration of local crop residues as a medium for growing oyster mushroom
- Demonstration of a recently released HYV of YLB *viz*., Vellayani Jyothika
- High density planting of tissue culture nendran banana
- Demonstration of high yielding varieties of black pepper
- Popularization of pot culture of bush pepper using popular variety Karimunda following organic PoP (Fig 14.1)
- Integrated disease management of phytophthora foot rot of black pepper
- Production of quality seed material of ginger
- Production of quality seed material of turmeric
- Cage culture of fishes in large water bodies
- Popularization of live feed for rearing ornamental fishes
- Fertility in anoestrum cows following CIDR treatment
- Demonstration of integrated farming systems for small and medium farmers

Fig 14.1 Popularizing bush pepper

OFT Programmes

These programmes aim at testing the new technologies developed at research stations in the field of crop husbandry, horticulture, animal husbandry, fisheries etc. to ensure their suitability and sustainability to the specific locations and to suggest or modify or refine the technology accordingly. The major OFT programmes carried out during the period are:

- Assessment of performance of high quality fodder grass *Thumburmuzhi-I* in upland condition
- Management of root mealy bug in banana
- Management of pseudostem weevil in banana
- Management of foot rot of black pepper
- Effect of bio stimulation of estrus induction and conception rate in crossbred heifer
- Effect of biostimulation on lactation of milk yield in dairy cattle
- Seed production of freshwater fishes
- Assessment of arecanut harvester
- Preparation of nutmeg rind candy
- Induction of flowering in Olour mango through paclobutrazol application combined with INM and IPM

Sponsored training programme - 'Friends of coconut'

'Friends of coconut' is a training programme which is aimed at providing employment opportunities for the unemployed youth. It was organized in collaboration with Coconut Development Board, Cochin. The main focus of the programme to introduce a new technique for climbing coconut trees with the help of a machine in addition to training on seed nut selection, nursery management, identification of disease and pest and their management. The programme consisted of 8 training schedules each schedule consisting of 20 unemployed rural youth aged between 18-40. Each schedule consisted of 6 days. There were 8 batches comprising 159 trainees, out of this 25 were women (Fig 14.2).

Revolving fund programme

The Kendra has a strong revolving fund programme to generate income for productive uses. Under this programme, quality planting materials of various crops are produced and made available to public at affordable rates. Income also was generated by way of sale of layer chicks, goats, heifers and bulls and consultation and doorstep

Fig 14.2 Training on Friends of Coconut for women

services through the clinic. During the period, an amount of ₹ 20.79 lakh has been realised through sale of planting materials, bioproducts, bioagents and the activities of Plant and Animal Health Centre.

Plant and animal health centre

The Kendra operates a plant and animal clinic offering various services to the farmers. An artificial insemination facility is also available at the centre to upgrade the genetic stock of livestock. The centre offers consultation, treatment and doorstep services with a nominal fee. In addition to the various treatments, the centre also provides vaccination facility and organises animal health camps in association with the state animal husbandry department. The various activities taken up by the clinic during the period are furnished below:

Consultancy/advisory /home service	- 640
Artificial insemination	- 329
Animal health campaigns/infertility camps	- 5
Vaccination of poultry birds and animals	- RDV-27800, IBD-26800, FMD-1255
Block *ksheeroltsavam*	- 5





Publications

Extension literature was published on *Trichoderma* against fungal diseases, *Pseudomonas* for protection of crops and Handbook/Training manual on scientific production and propagation of spices.

Participatory seed production in ginger and turmeric

KVK has identified 20 potential turmeric and ginger farmers in Kozhikode and supervised at field level for scientific seed production. Good quality seed material produced was assembled at KVK and sold to need farmers. A total of 438 kg turmeric IISR Prabha and 325 kg of ginger IISR Varada were sold to 110 farmers.

Kisan Mobile SMS Service: KVK started short message Service (SMS) to all registered farmers on latest updates in agriculture and allied fields over their mobile phones. The SMS are being sent to farmers regarding new interventions, latest technologies, market price of agriculture produce, weather forecast, disease management measures, planting material availability forthcoming trainings etc. KVK has so far sent 41 SMS to farmers over the district.

ICAR best KVK Award

The KVK was also conferred with the ICAR best KVK Award 2011 for Zone VIII for its outstanding contributions (Fig 14.3) which includes a certificate, citation, and cash prize of ₹ 4 lakhs.


Indian Council of Agricultural Research

is presented to

Krishi Vigyan Kendra, Kozhikode

Calicut 673528

for their outstanding contribution in

Extension Education

Fig 14.3 ICAR Best KVK award certificate

15. RESEARCH PUBLICATIONS

1. Anandaraj M, Dinesh R, Srinivasan V and Hamza S 2012 Nanotechnology in Agriculture: the use of novel materials and environmental issues. The Botanica 59-61: 22-34.

2. Balasubramanian S, AM Mohite, Singh KK, John Zachariah T and Anand T 2012 Physical properties of turmeric (*Curcuma longa* L). Journal of Spices and Aromatic Crops 21:178-181.

3. Balasubramanian S, Singh KK, Ashish GR, Mohite AM and John Zachariah T 2012 Physical properties of cinnamon bark. Journal of Spices and Aromatic Crops 21:161-163.

4. Bhat AI, Siljo A and Devasahayam S 2012 Occurrence of symptomless source of Piper yellow mottle virus in black pepper (*Piper nigrum* L.) varieties and a wild Piper species. Archives of Phytopathology and Plant Protection 45: 1000-1009.

5. Hamza S, Leela NK, Srinivasan V, Nileena CR and Dinesh R 2013 Effect of Zinc fertilization on quality profile of ginger. Journal of Spices and Aromatic Crops 22: 93-96.

6. Jayashree E and Visvanathan R 2012 Development of hand-operated mechanical ginger peeler. Journal of Horticultural Sciences 7: 75-80.

7. Jayashree E and Visvanathan R 2012 Thin layer drying of ginger (*Zingiber officinale*) in a multi-rack type solar tunnel drier. Indian Journal of Agricultural Sciences 82: 351–355.

8. Jayashree E, Visvanathan R and John Zachariah T 2012 Quality of dry ginger (*Zingiber officinale*) by different drying methods. Journal of Food Science and Technology. DOI 10.1007/s13197-012-0823-8.

9. Kumar A, Prameela TP and Suseela Bhai R 2013 A unique DNA repair and recombination gene (recN) sequences for identification and intraspecific molecular typing of bacterial wilt pathogen, *Ralstonia solanacearum* and its comparative analysis with ribosomal DNA sequences. Journal of Biosciences 38: 267-278.

10. Leela NK, Pervez R, Ramana KV, Rosana OB and Eapen SJ 2012 Nematicidal activity of *Strychnos nuxvomica* leaf and its constituents against the root-knot nematode, *Meloidogyne incognita.* Nematol medit. 40: 116-123.

11. Pervez R, Eapen SJ and Devasahayam S 2012 Adrak ko hani phochaney wale ketoon ka ketnashak sutkrimi duara Niyantaran. Bhartiya Krishi Anusandhan Patrika, 27: 228-230.

12. Pervez R, Eapen SJ, Devasahayam S and Jacob TK 2012 Efficacy of some entomopathogenic nematodes against insect pests of ginger and their multiplication. Nematol medit. 40: 39-44.

13. Pervez R, Eapen SJ, Devasahayam S and Jacob TK 2013 A new species of entomopathogenic nematode *Oscheius gingeri* sp. n. from ginger rhizosphere. Archieves of Phytopathology and Plant Protection, 46: 526-535.

14. Pervez R, Leela NK and Eapen SJ 2012 Nematicidal activity of *Strychnos nuxvomica* leaf and its constituents against the *Radopholus similis* infesting black pepper (*Piper nigrum* L.). Current Nematology, 22: 49-53.





15. Sajini AV, Kandiannan K, Srinivasan V, Saji KV and Thankamani CK 2012 Weed flora of black pepper garden at high rain fall tract of northern – agroclimatic zone of Kerala. Journal of Plantation Crops 40: 75-81.

16. Sangeeth K, Suseela Bhai R and Srinivasan V 2012 *Paenibacillus glucanilyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. Journal of Spices and Aromatic Crops 21: 118-124.

17. Simi Mohan, Utpala Parthasarathy and Nirmal Babu K 2012 *In vitro* and *in vivo* adventitious bud differentiation from mature seeds of three garcinia spp. Indian Journal of Natural Products and Resources 3: 65-72.

18. Simi Mohan, Utpala Parthasarathy, Asish GR and Nirmal Babu K 2012 Evaluation of genetic stability of micropropagated plants of three species of Garcinia using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Indian Journal of Biotechnology 11: 341-343.

19. Suseela Bhai R, Sasikumar B and Kumar A 2012 Evaluation of ginger germplasm for resistance to soft rot caused *Pythium myriotylum*. Indian Phytopathology 65: 93.

20. Thankamani CK, Kandianan K, Madan MS, Raju VK, Hamza S and Krishnamurthy KS 2012 Feasibility of intercropping medicinal plants in black pepper garden. Indian Journal of Spices and Aromatic plants 21: 113-117.

21. Thankamani CK, Kandiannan K and Hamza S 2012 Intercropping medicinal plants in black pepper. Indian Journal of Horticulture 69: 133-135.

22. Utpala Parthasarathy, Nandakishore OP, Senthil Kumar R, Nirmal Babu K, Zachariah TJ and Parthasarathy VA 2012 Chromatographic fingerprinting and estimation of organic acids in selected Garcinia species. International Journal of Innovative Horticulture, 1: 68-73.

23. Utpala Parthasarathy, Nirmal Babu K, Senthil Kumar R, Ashis GR, Mohan S and Parthasarathy VA 2013 Diversity of Indian garcinia - a medicinally important spice crop in India. Acta Horticulturae 979: 467-476.





16. EDUCATION AND TRAINING

Trainings conducted

- Cheminformatics- Tools and Applications from 19 to 22 February 2013
- Next Generation Sequencing- data analysis and annotation from 12 to 16 March 2013
- One month summer training on biochemistry, biotechnology and bioinformatics for eight M.Sc. students from 8 May to 6 June 2012

M.Sc.

- One student completed post M.Sc. training

Ph.D.

1. *Siju Senan*, Applied Botany, 'Development of microsatellite markers in *Curcuma longa* L. and its cross-species amplification', Mangalore University
2. *Asish G R*, Biochemistry, "Biochemical Molecular and Spatial (GIS) Variability in Garcinia species with special reference to *Garcinia gummigutta* and *Garcinia indica*", Mangalore University
3. *Jiby Mary Varghese*, Applied Botany, "Agrobacterium mediated transformation of black pepper using sequences from Cucumber mosaic virus and Piper yellow mottle virus", Mangalore University
4. *Anoop K,* Applied Botany, "Etiology and disease management of Rhizome rot in Turmeric (*Curcuma longa* L.)", Mangalore University
5. *Sangeeth K P,* Applied Botany, "Development and Formulation of Effective Biofertilisers for Management of Black pepper and Cardamom", Mangalore University

Officials attended	Training programme	Date	Organization
T Arumuganathan	Human resource management for extension personnel	23-27 April 2012	MANAGE, Hyderabad
Rashid Pervez Prasannakumari	Unicode training programme	12 July 2012	Town Official Language Implementation Committee, Kozhikode
P V Sali Mr. K Faisal	Developing executive secretaries, personal assistants & office staff for future	11-15 June 2012	National Productivity Council, New Delhi
R Praveena	Short course on plant disease diagnostics: theory and practices	4-13 July 2012	CPRI, Shimla
D Prasath	Intellectual property rights and biotechnology	17-23 July 2012	NAARM, Hyderabad
R N Subramanian V V Sayed Mohammed	Programme on inventory management	23-25 July 2012	Administrative Staff College of India, Hyderabad
T Arumuganathan	Geospatial technologies and application	22 August to 11 September 2012	Department of Remote Sensing and GIS, TNAU, Coimbatore
N K Leela E Jayashree	Food safety and supply chain management in spices and botanical ingredients	17-21 September 2012	Spices Board, Kochi
Utpala Parthasarathy	Computational genomic analysis technology in discovery of agronomically important crop genes	24-29 September 2012	NBPGR, New Delhi
C K Thankamani	National training on advances in weed management	31 October to 9 November 2012	Directorate of Weed Science Research, Jabalpur
S Hamza	Communication and presentation skills	19-23 November 2012	IMTR, GOA
Johnson George K	Intellectual property and PPVFR Act, 2001	22-24 November 2012	Scriboard Research & Development Centre, New Delhi
K M Prakash	Advances in arecanut and cocoa production technology	3-9 December 2012	CPCRI, Regional station, Vittal
R Suseela Bhai N K Leela	Training programme on communication and presentation skills for women scientists-sponsored by DST	7-11 January 2013	IMTR, Goa
V K Abubacker Koya M K Raveendran	Nursery management	20-22 February 2013	Central Arid Zone Research Institute, Jodhpur









17. INSTITUTE TECHNOLOGY MANAGEMENT UNIT

- The unit has facilitated Non-exclusive license agreements in turmeric and ginger varieties, IISR Prathibha and IISR Varada with National Horticultural Research and Development Foundation (NHRDF), with Katra Phyto Chem. Pvt. Ltd, Bangalore for IISR Prathiba and with Mr. Tom C Antony, Cheripurathu Nursery, Kottayam for nutmeg variety IISR Viswashree (Fig 17.1).
- Six formulations of crop specific micronutrient mixtures developed are in the process of commercialization and patenting. Seed dressing technology for seed spices, microbial consortium for black pepper, PGPR talc formulations for ginger and diagnostics for virus detection in black pepper are also in the process of commercialization through National Research Development Corporation (NRDC).
- The invention entitled "Bacterial fermentation technology for production of high quality off-odour-free white pepper from matured green pepper (*Piper nigrum* L.)" was granted patent (Application No.3433/CHE/2011 A; dated 20/04/12).
- A novel technology for delivery of PGPR is in process for patent filing and commercialization.
- Released variety of cardamom, Appangala-1 was approved for registration as extant variety by the PPV&FRA (Registration No. 134/2012). Ten other varieties are in the process of approval by PPV & FRA. A book entitled "IPR: Current Scenario in Spices" was published.

Fig 17.1. License issued to Mr. Tom C Antony, Kottayam by Director, IISR for nutmeg variety IISR Viswashree

18. HINDI CELL ACTIVITIES





OLIC meeting

The Official Language Implementation Committee (OLIC) meets once in every quarter; First on 11th May 2012; second on 4th August 2012; third on 30th November 2012 and forth on 8th March 2013 under the chairmanship of Dr. M. Anandaraj, Director and reviews the official language implementation activities of the institutes.

Workshops conducted

Four workshops were organized at IISR, Calicut (i) How to popularize official language implementation in the office" on 23rd May 2012 (ii) Noting and drafting on 19th September' 2012 (iii) Noting and drafting on 12th December' 2012 and (iv) Hindi translation and words pronunciation on 18th March 2013 (Fig 18.1).

Hindi day and Hindi week celebration

Hindi Day was celebrated on 14th September 2012 and Hindi Week from 17th- 22nd September 2012. Hindi week inauguration was held on 17th September 2012 under the president ship of Dr. M. Anandaraj, Director. During this week various competitions *viz.*, extempore speech, song, debate, noting and drafting, memory test, caption writing and *anthakshari* were conducted for the staff members and prizes were distributed to the winners in the valedictory function on 22st September 2012. Dr. H. C. Joshi, Director (OL), ICAR, New Delhi was the chief guest. Institute official language magazine, *Masaloon ki Mehak* also release on this occasion (Fig 18.2).

TOLIC activity

Dr. S. Devasahayam, Head, Division of Crop Protection, Dr. Rashid Pervez, Senior Scientist & Hindi Officer and Ms. N. Prasannakumari, Hindi translator attended the 49th TOLIC meeting on 25th April 2012 at King Fort Hotel, Kozhikode. Mr. B. Krishnamoorthy, Dr. Rashid Pervez and Ms. N. Prasannakumari attended the 50th TOLIC meeting and present institute OL implementation report by Dr. Rashid Pervez on 24th September 2012 at Hotel Malabar, Kozhikode. Dr. Rashid Pervez and Ms. N. Prasannakumari attended subcommittee meeting of TOLIC at SBT, Kozhikode on 12th July 2012 and 23rd January 2013 and organized valedictory function of Joint Hindi Fortnight of TOLIC on 7th Feb., 2013 at Regional Science Centre, Kozhikode.

Inspection

Dr. P. R. Rao, Deputy Director (OL), Indian Council of Agricultural Research, New Delhi inspected official language implementation activities in the institute on 8th November 2012.

Publications

Masala Samachar (4 issues), Annual report (2011-12), *Anusandhan ke mukhya ansh* (2011-12), Executive summary of annual report of the institute and AICRPS (Spices), *Rashtrya ki unnatee mein Masaloon ka yogdan, Masaloon ki Mehak* (OL magazine) and 8 popular scientific hindi articles were published. Quarterly and annual reports on official language activities of the institutes were prepared and sent to ICAR, New

Delhi, TOLIC, Calicut and Regional Implementation Office, Cochin. The half yearly report on Official Language implementation has been prepared and submitted to Regional Implementation Office, Cochin.

Other activities

Various Office orders, circular, documentaries, rubber stamps, name board, envelops and web site were translated into Hindi. Daily a word/ phrase in Hindi and its transliteration in English was displayed on the notice board.

Fig 18.1 Hindi Workshop

Fig 18.2 Hindi Week valedictory function

19. RECOGNITION





Indian Institute of Spices Research, Kozhikode received two awards for the official language implementation in the 51st meeting of the Town Official Language Implementation Committee at Malabar Hotel, Kozhikode on 25th April 2013.

Rajbhasha shield award- 2012 (First prize)

This award was granted for significant contribution to Hindi correspondence, organizing hindi workshops, meetings, publications like, Annual Report, AICRPS Annual Report, Research Highlights, News Letter, Popular Articles, Official Language Magazine, Extension Bulletins in hindi and OL implementation during 2011-12 (Fig 19.1).

Official language magazine award -2012 (First Prize)

This award was granted for official language magazine *Masaloon ki Mehak* , among the 73 central government organizations and members of the TOLIC, Kozhikode. Dr. M. Anandaraj, Director was the Patron and Dr. Rashid Pervez, Senior Scientist and Hindi Officer was the Chief Editor of the magazine.

Fig 19.1 Mr. B. Krishnamoorthy, Head I/C Crop Improvement division and Dr. Rashid Pervez, Senior scientist and Hindi officer receive Rajbhasha Shield Award.

20. INSTITUTE MANAGEMENT COMMITTEE





1.	Dr. M. Anandaraj , Director, IISR, Kozhikode	Chairman
2.	Director of Agriculture, Govt. of Kerala, Thiruvananthapuram	Member
3.	Director of Horticulture, Govt. of Tamil Nadu, Tamil Nadu	Member
4.	Dr. B. Raju, Director of Education (Dean of the University) & Dean, Postgraduate Studies, University of Horticulture Sciences, Bagalkot-587 102	Member
5.	Sri. P. Brahmaiah, Senior Finance & Accounts Officer, Central Plantation Crops Research Institute, Kasaragod-671 124	Member
6.	Sri. Sulfikar Mayoori, Mayoori, Vaidyar Veedu, Kayamkulam, Alappuzha	Member
7.	Sri. Adv. C.V. Damodharan, Kattukulangara, Anandashram, P.O. Kanhangad, Kasaragod.	Member
8.	Mr. V. Mohanan, Administrative Officer, IISR, Kozhikode	Secretary

LIST OF PROJECTS





I. Institute Projects:

Mega Project I: Breeding improved varieties of spice crops for yield, quality, drought and resistance to pests and diseases [Project Leader: B Krishnamoorthy]

1. Gen. X (813): Breeding cardamom for high yield and disease resistance (2007-2015) [R Senthil Kumar, R Praveena and C M Senthil Kumar]
2. Gen. XXV (813): Genetics of seedling progenies of turmeric (*Curcuma longa* L.) (2007-2013) [R Ramakrishnan Nair]
3. Gen. XXIX (813): A comparative study of molecular and biochemical diversity of Garcinia of Eastern Himalayas and Western Ghat ranges with GIS (2008-2014) [Utpala Parthasarathy and R Senthil Kumar]
4. Gen. XXX (813): Evaluation of genetic variability in vanilla with emphasis to disease tolerance (2010-2015) [R Ramakrishnan Nair]
5. Gen. XXXI (813): Breeding black pepper for high yield, quality and resistance to stresses (2012-2017) [B Sasikumar, Johnson K George, K V Saji, T E Sheeja, T John Zachariah, R Suseela Bhai, K S Krishnamuthy and S Devasahayam]
6. Gen. XXXII (813): Expression profiling and allele mining of genes induced under water-deficit stress in black pepper (*Piper nigrum* L.) (2012-15) [Johnson K George and K S Krishnamurthy]
7. Biotech. XII (813): Mining of DNA markers and Genes from Expressed Sequence Tags of *Curcuma longa* (2012-2015) [Sheeja TE and B Sasikumar]
8. Gen. XXXIV (813): Induction of variability in ginger through induced mutation for yield and disease resistance (2012-2017) [D Prasath, R Ramakrishna Nair and R Suseela Bhai]

Mega Project II: Collection, conservation, characterization and cataloguing of germplasm of spice crops for yield and other economically important characters [Project Leader: P.A. Mathew]

1. Gen. XXVIII 813: Conservation and characterization of *Piper* germplasm (2008-2014) [K V Saji, R Senthil Kumar and P Umadevi]
2. Gen. XIX (813): Conservation, characterisation, evaluation and improvement of *Zingiber* and *Curcuma* Sp. (2007-2015) [D Prasath, B Sasikumar and K V Saji]
3. Gen. XXVI (813): Evolving high yielding and high quality nutmeg clones by selection (2007-2016) [B Krishnamoorthy and J Rema]
4. Gen. XXXIII (813): Identification of core collection, characterization and maintenance of cardamom germplasm (2012-2017) [R Senthil Kumar, S J Ankegowda and C N Biju]

Mega Project III: Production physiology of spice crops [Project leader: Dr. S.J. Ankegowda]

1. Phy. X (813): Evaluation of black pepper and cardamom elite lines for yield and quality under moisture stress (2010–2015) [S J Ankegowda and K S Krishnamurthy]
2. Phy. XI (813): Source-sink relationship, endogenous hormone levels and their relationship with rhizome development in ginger and turmeric (2011-2014) [K S Krishnamurthy and K Kandiannan]

Mega Project IV: System approach for sustainable production of spices [Project Leader: Dr. R. Dinesh]

1. SSC V (813): Studies on allelopathy in tree species-black pepper interactions (2009-2013) [R Dinesh and S Hamza]
2. SSC VI (813): Nutrient cycling and soil C sequestering potential of spice crops under different management systems (2011-2015) [V Srinivasan, R Dinesh, S J Ankegowda and S Hamza]
3. Agr. XXIX (813): Effect of weed management practices on growth, yield and quality parameters of ginger (2011-2014) [C K Thankamani and K Kandiannan]
4. Hort. VII (813): Evaluation of nutmeg for its suitability for high density planting (2011-2016) [J Rema and PA Mathew]

Mega Project V: Secondary agriculture and utilization of high value compounds in spices [Project Leader: T. John Zachariah]

1. PHT. V (813): Studies on improved processing and quality evaluation of major spices (2010-2013) [E Jayashree, N K Leela and T Arumughanathan]
2. PHT. VI (813): Studies on production of food extrudates from selected spices (2011-14) [E Jayashree, T John Zachariah and Thajudeen Sheriff (CTCRI)]
3. Org. Chem. III: Flavour profiling of Zingiberaceae spices (2008-2013) [N K Leela and S Hamza]
4. Biochem. VIII (813): Evaluation of spice extracts for anticancer effect in relation to telomerase activity (2012-2016) [B Chempakam and K Sujathan (RCC, Thiruvananthapuram)]

Mega Project VI: Propagation studies in spice crops [Project Leader: K. Kandiannan]

1. Hort. V (813): Rootstock intervention to manage root infection of Phytophthora and nematodes in black pepper (2006-2013) [P A Mathew]

Mega Project VII: Identification, characterization and development of diagnostics against pests, pathogens and nematodes of spice crops [Project Leader: A Ishwara Bhat]

1. Path. XXI (813): Diversity of rhizome – root rot pathogens and their antagonists in cardamom. (2010 – 2014) [R Praveena and C N Biju]
2. Path XXII (813): Investigations on the endophytic and rhizospheric microflora associated with cardamom and allied genera (2012-15) [C N Biju and R Praveena]
3. Ent. XIV (813): Survey and documentation of naturally occurring entomopathogens in spice cropping systems (2012-2015) [C M Senthil Kumar, T K Jacob and S Devasahayam]

Mega Project VIII: Identification and characterization of pest, pathogen and nematode resistance in spice crops [Project Leader: R Suseela Bhai]

1. Ent. XIII (813): Screening of germplasm accessions of spices and evaluation of antibiosis resistance to major insect pests (2006-2013) [T K Jacob, S Devasahayam and C M Senthil Kumar]
2. Path. XX (813): Screening of *Piper* germplasm accessions against *Piper* yellow mottle virus (PYMoV) (2008-2015) [A Ishwara Bhat, T K Jacob and K V Saji]
3. Nema. IV (813): Role of phenyl propanoids in black pepper - burrowing nematode interactions (2008-2013) [Santhosh J Eapen]

Mega Project IX: Developing integrated pest and disease management strategies in spice crops. [Project Leader: S Devasahayam]

1. Crop. Prot. 1.5 (813): Integrated management of *Phytophthora* foot rot and slow decline diseases of black pepper (2008-2014) [R Suseela Bhai, Santhosh J Eapen and Rashid Pervez]
2. Nema. VI (813): Mass production and field evaluation of promising entomopathogenic nematodes against insect pests infesting major spices (2012-2016) [Rashid Pervez, Santhosh J Eapen and S Devasahayam]

Mega Project X: Transfer of technology and impact assessment [Project Leader: P. Rajeev]

1. Ext. IV(813) : Training of research and extension personnel (2005-2013) [P. Rajeev]
2. Ext. V (813): A Study on diffusion, adoption and impact of varieties released from IISR and scientific crop management practices (2006-13) [P Rajeev]

Mega Project XI: Developing customized software and expert-system on spices [Project Leader: S J Eapen]

1. Stat. I (813): Development of databases and software (2004-2013) [K Jayarajan]
2. Agr XXX (813): Database on agro-technologies generated for major spices (Black pepper, Cardamom, Ginger and Turmeric) in agro-climatic regions of India (2011 -2014) [K Kandiannan, Utpala Parthasarathy, K Jayarajan and CK Sushamadevi]

II. Externally aided Projects:

i) Department of Biotechnology, New Delhi

1. DBT Twinning Programme for the NE: Seed system development in major spice crops (Ginger, turmeric and Naga Chilli) of NER through *in vitro* techniques (2012-2015) [K Nirmal Babu and K. Kandiannan]
2. DBT-CIB-5: Development of comprehensive SSR and SNP markers for the study of genetic diversity and association analysis in *Curcuma* (2012-2015) [T E Sheeja, D Prasath and B Sasikumar]
3. DBT-CP-4: Accredited Test Laboratory (ATL) under the national certification system for tissue culture raised plants (NCS-TCP) (2008-2013) [A Ishwara Bhat and K Nirmal Babu]
4. DBT-CP6: Genome mining of spice associated endophytic bacteria for natural products (2011-2014) [Santhosh J Eapen and R Suseela Bhai]
5. DBT-CP5: Testing transgenic black pepper for resistance to viruses (2011-2014) [A. Ishwara Bhat and D. Prasath]
6. DBT-SS1: Distributed Information Sub-Centre (2000-2013) [Santhosh J Eapen]









ii) Indian Council of Agricultural Research, New Delhi

1. ICAR-CPPHT-1: Network project on organic farming (2007-2013) [V Srinivasan, C K Thankamani and T John Zachariah]
2. ICAR Mega Seed Project: Production of nucleus planting materials of improved varieties of spice crops (2006-2013) [K Kandiannan, P A Mathew and S J Ankegowda]
3. ICAR-CP 4: Application of microorganisms for agriculture and allied sectors (AMAAS): Nutrient management, PGPR and biocontrol (2007-2013) [M Anandaraj, R Dinesh and N K Leela]
4. Outreach Programme on *Phytophthora, Fusarium & Ralstonia* diseases of horticultural and field crops (2008-2013) [M Anandaraj, R Suseela Bhai, Santhosh J Eapen, K Nirmal Babu, Johnson K George and D Prasath]
5. Outreach Programme on management of sucking pests in horticultural crops: (2009-2013) [T K Jacob, S Devasahayam and C M Senthil Kumar]
6. Outreach Programme on diagnosis and management of leaf spot diseases infield and horticultural crops (2009-2013) [C N Biju and R Praveena]

iii) Ministry of Food Processing Industries, New Delhi

1. MoFPI-CIB-1: DNA barcoding to discriminate traded spices from their adulterants (2012-2014) [B Sasikumar and T E Sheeja]

iv) Department of Information & Technology, New Delhi

1. DoE-CPPHT-1: Developing electronic nose for monitoring cardamom aroma (2012-13) [N K Leela and Nabarun Bhattacharya]

v) Department of Science and Technology, New Delhi

1. DST-CPPHT-1: Development of mechanical unit for production of white pepper from green pepper (2012-13) [E Jayashree, R Suseela Bhai, T John Zachariah and Ravindra Naik (RRL)]

vi) National Agricultural Innovation Project, New Delhi

1. NAIP-CPPHT-1: Studies on cryogenic grinding for retention of flavour and medicinal properties of some important Indian spices (2009-2013) [T John Zachariah and N K Leela]
2. NAIP SS-II: Mobilizing mass media support for sharing agro-information (2009-2013) [T J Zachariah, P Rajeev and T K Jacob]

vii) State Planning Board, Govt. of Kerala, Thiruvananthapuram

1. Kerala State-CPPHT-1: Soil based nutrient management plans for agro ecosystems of Kerala (2010-2013) [R Dinesh, V Srinivasan and S Hamza]
2. Kerala State – CPPHT-2: Pepper rehabilitation package – technology mission on black pepper for Wayanad – SUGANDHI (2010-2013) [V Srinivasan, T K Jacob, R Suseela Bhai, R Dinesh, C K Thankamani, K Kandiannan, A Ishwara Bhat, Santhosh J Eapen, S J Ankegowda, Rashid Pervez, K S Krishnamurthy, P Rajeev, C N Biju and S Hamza]

PERSONNEL

Headquarters

Scientific

Sl.No.	Name	Designation
1	Dr. M. Anandaraj	Director
2	Dr. K. Nirmal Babu	Project coordinator AICRPS - from May 2012
3	Dr. S. Devasahayam	Head, Crop Protection Division
4	Dr. T. John Zachariah	Head, Crop Production & Post Harvest Technology
5	Mr. B. Krishnamoorthy	Head (i/c), Crop Improvement
6	Dr. B. Chempakam	Principal Scientist (Biochemistry)
7	Dr. B. Sasikumar	Principal Scientist (Plant Breeding)
8	Dr. T.K. Jacob	Principal Scientist (Entomology)
9	Dr. J. Rema	Principal Scientist (Horticulture)
10	Dr. Johnson K. George	Principal Scientist (Genetics & Cytogenetics)
11	Dr. C.K. Thankamani	Principal Scientist (Agronomy)
12	Dr. R. Dinesh	Principal Scientist (Soil Science)
13	Dr. R. Suseela Bhai	Principal Scientist (Plant Pathology)
14	Dr. A. Ishwara Bhat	Principal Scientist (Plant Pathology)
15	Dr. R. Ramakrishnan Nair	Principal Scientist (Gen. & Cytogenetics)
16	Dr. K.S. Krishnamurthy	Principal Scientist (Plant Physiology)
17	Dr. K. Kandiannan	Principal Scientist (Agronomy)
18	Dr. N.K. Leela	Principal Scientist (Organic Chemistry)
19	Dr. Santhosh J. Eapen	Principal Scientist (Nematology)
20	Dr. K.V. Saji	Principal Scientist (Economic Botany)
21	Dr. P. Rajeev	Senior Scientist (Agril. Extension)
22	Dr. V. Srinivasan	Senior Scientist (Soil Science)
23	Dr. A. Shamina	Senior Scientist (Bio chemistry) – till December 2012





24	Dr. T.E. Sheeja	Senior Scientist (Biotechnology)
25	Dr. Rashid Pervez	Senior Scientist (Nematology)
26	Dr. D. Prasath	Senior Scientist (Horticulture)
27	Dr. C.M. Senthilkumar	Senior Scientist (Entomology) – from April 2012
28	Dr. E. Jayashree	Scientist, Senior Scale (AS & PE)
29	Ms. P. Umadevi	Scientist (Biotechnology)

Technical Officers

1	Dr. Johny A. Kallupurackal	Technical Officer (T9)
2	Dr. Hamza Srambikkal	Technical Officer (Lab) (T9)
3	Dr. Utpala Parthasarathy	Technical Officer (T9)
4	Mr. K. Jayarajan	Technical Officer (Statistics) (T6)
5	Dr. C.K. Sushama Devi	Technical Officer (T6) (Library)
6	Ms. N. Prasannakumari	Technical Officer (T6) (Hindi Translator)
7	Mr. K.T. Muhammed	Technical Officer (T5) (Farm)
8	Mr. V. Sivaraman	Technical Officer (T5) (Farm)
9	Mr. A. Sudhakaran	Technical Officer (T5) (Artist-cum-Photographer)
10	Mr. N.A. Madhavan	Technical Officer (T5)

Administrative

1	Mr. V.L. Jacob	Finance & Accounts Officer - from May 2012
2	Mr. V. Mohanan	Administrative Officer
3	Mr. K.G. Jegadeesan	Assistant Finance and Accounts Officer
4	Mr. C. Venugopalan	Assistant Administrative Officer
5	Mr. R.N. Subramanian	Assistant Administrative Officer
6	Ms. P.V. Sali	Private Secretary

IISR Experimental Farm, Peruvanamuzhi

Scientific

1	Mr. P.A. Mathew	Principal Scientist (Horticulture)

Technical Officer

1	Mr. V.K. Aboobacker Koya	Farm Superintendent (T9)
2	Mrs. E. Radha	Technical Officer (T7-8)
3	Mr. K. Kumaran	Technical Officer (T5)

Krishi Vigyan Kendra

Scientific

1	Dr. T. Arumuganathan	Programme Coordinator

Technical Officer

1	Mr. P.S. Manoj	(T9) (Horticulture)
2	Dr. S. Shanmugavel	(T9) (Veterinary Science)
3	Mr. K.M. Prakash	(T9) (Agronomy)
4	Dr. B. Pradeep	T6 (Fisheries)
5	Ms. A. Deepthi	T6 (Home Science)
6	Mrs. K K Aiswariya	T6 (Plant Protection)

IISR Cardamom Research Centre, Appangala

Scientific

1	Dr. S.J. Ankegowda	Principal Scientist (Plant Physiology)
2	Dr. R. Senthil Kumar	Senior Scientist (Horticulture)
3	Dr. C.N. Biju	Scientist (Plant Pathology)
4	Dr. R. Praveena	Scientist (Plant Pathology)

Administrative

1	Mr. P. Muraleedharan	Assistant Administrative Officer

WEATHER DATA 2012





Cardamom Research Centre, Appangala

	Temperature (°C)		Rainfall	
Months	Maximum	Minimum	(mm)	Rainy days
January	28.1	10.1	0.0	0
February	29.6	11.7	0.0	0
March	31.8	14.5	5.0	1
April	30.5	17.2	167.0	11
May	28.0	18.2	36.9	1
June	26.8	17.2	311.5	19
July	25.6	18.3	396.8	31
August	24.3	17.4	649.6	25
September	26.0	18.3	249.6	17
October	30.5	17.4	63.9	8
November	30.8	16.3	155.8	4
December	33.7	14.2	13.0	1
Average/Total	**28.81**	**15.9**	**2049.1**	**118**

IISR Experimental Farm, Peruvannamuzhi

	Temperature (°C)		Rainfall	
Months	Maximum	Minimum	(mm)	Rainy days
January	34.3	20.3	0.0	0
February	35.2	18.5	0.0	0
March	35.1	21.2	28.0	1
April	35.5	22.1	178.4	16
May	33.9	24.0	105.2	2
June	29.1	22.1	636.4	23
July	28.1	21.4	742.8	27
August	27.8	21.7	879.5	26
September	29.0	21.1	446.8	15
October	31.4	21.7	387.5	15
November	31.5	20.5	359.0	10
December	34.1	19.8	0	0
Average/Total	**32.08**	**21.2**	**3763.6**	**135**

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