Research Highlights 2012-13

Indian Institute of Spices Research
Kozhikode

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PREFACE

The summary of our achievements for the year 2012-13 is presented here as Research Highlights. During this year, collections were made to enrich the cultivar diversity 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. Encapsulated bead and liquid formulations of biocontrol agents have been standardized for exploiting its commercial potential. 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, which is cost effective and does not need any sophisticated equipments. A SYBR Green based method for real-time quantitative RT-PCR (qPCR) 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 are being 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 Farmer's - Scientists interaction meet were organized at Cardamam Research Centre (CRC), Appangala during the third week of December 2012. About 1700 soil samples from farmer's plots have been analyzed for all the essential nutrients and soil health cards with nutrient advisories were given. FLDs on varieties and technologies of black pepper were conducted at Wayanad district. The KVK conducted nine seminars, participated in eight Kisan Melas or exhibitions and trained more than 5150 beneficiaries. Participatory seed production on high yielding varieties of ginger and turmeric was taken up in ten 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 participated in three national and four state level exhibitions, organized training programmes to about 100 horticultural officers and farmers of Uttar Pradesh, Andhra Pradesh, Arunachal Pradesh, Karnataka and Kerala, conducted media visits to progressive farmer's plots and popularized the technologies 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, 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 publication.

Kozhikode
20.03.2013

M Anandaraj
Director

CONTENTS

BLACK PEPPER

CROP IMPROVEMENT

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 three wild *Piper* species. Spike proliferating cultivar *Thekkan*, other farmer selected cultivars such as *Kumbakkal* and *Ponmani* are some of the unique accessions collected from Idukki district (Fig 1a&b). A drought tolerant type was collected from Malappuram district (Fig 1c). The present status of black pepper germplasm conserved is 2936 accessions. Acc. 7398, a cultivated black pepper having spike length of 27.3 cm (Fig 1d), but with poor setting was collected from Yemma Gundi Estate, Suntikoppa, Madikeri. This accession was found to have the longest spike length (average of 26.5 cm) among the black pepper accessions collected so far.

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.

Fig 1a. Ponmani

Fig 1b. Kumbakkal

Fig 1c. A drought tolerant accession collected from Malappuram

Fig 1d. Acc.7398 with very long spike

Rootstock to manage infection of *Phytophthora* and nematodes

Grafting *Sreekara* variety of black pepper with interstocks of *P. colubrinum* and *P. hamiltoni* on *Piper* Acc. 5815 was not successful since the survival and growth were poor. The combination *P. ornatum* and *P. hamiltoni* + *Sreekara* that appeared promising showed poor growth and survival after one year. However, grafting of *P. ornatum* with *P. chaba* was successful and growth was found to be very good. *P. hamiltoni* as rootstock, though compatible with black pepper cannot be used under rainfed conditions since it is susceptible to drought. ISSR marker studies on mentor graft seedlings of *Panniyur – 1* on *P. colubrinum* stock were done but transfer of genetic material from *P. colubrinum* could not be established to account for the variation among the seedlings.

CROP PRODUCTION

Drought tolerance studies

Fifty germplasm accessions were screened for drought tolerance and the 6707 was identified as relatively tolerant based on its high relative water content (RWC) and low membrane leakage values even at 8 to 8.5 % soil moisture condition. Ten previously identified drought tolerant accessions along with CV *Subhakara* (check) have been planted in the field farm for further evaluation.

Organic production package developed

Organic production package involving 10 kg FYM + 500 g neem cake + 500 g ash+ 2 kg vermicompost with biofertilizers – *Azospirillum* and P solubilizing bacteria (20 g) and *Trichoderma* (50 g) and *Pseudomonas* (IISR 6) (50 g) per vine and spray of 1% Bordeaux mixture (BM) and neem oil (5 mL^{-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 tree species like *Garuga, Erythrina, Gliricidia, Ailanthus*, Jack and Silver Oak at 100% concentration was applied to bush pepper @100 mL 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 (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.

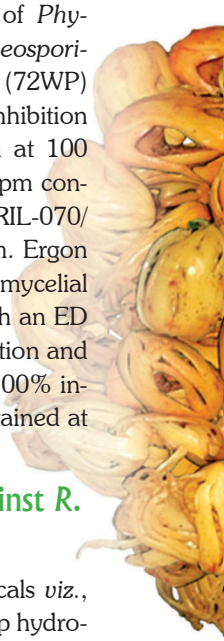
Microbial consortium for growth promotion

A consortium of three rhizobacterial isolates (BRB 3, BRB 13 and BRB 23) markedly promoted the growth of black pepper. However, the PGPR (BRB 3, 13 and 23) applied in combination with various rates of chemical fertilizers differed in their effects on black pepper 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 and highest number of leaves were obtained from 100% N + 100% P + 75% K + BRB3 + BRB13. Based on a series of experiments, microbial formulation involving the consortium of PGPR *viz*., *IISR Biomix* was developed for licensing and commercialization.

CROP PROTECTION

Evaluation of new chemicals against *P. capsici*

The anti-oomycetal 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 50 ppm, sporulation at 100 ppm and zoospore germination at 200 ppm concentrations. The average ED 50 value of RIL-070/FI (72WP) against *P. capsici* was 30 ppm. Ergon 44.3% (w/w) showed 100% inhibition of mycelial growth and sporulation at 6000 ppm with an ED 50 value of 210.6 ppm for mycelia inhibition and 1480.4 ppm for sporulation inhibition. 100% inhibition of zoospore germination was obtained at 1000 ppm.



Evaluation of new chemicals against *R. similis*

Nematicidal activity of eight chemicals *viz*., Fipronil, Thiamethoxan, Acephate, Cartap hydrochloride, Quinalphos, Flubendamide, Carbosulfan and Chloropyriphos were evaluated against *R. similis* infesting black pepper in the green house. Out of these five chemicals *viz*., Fipronil, Thiamethoxan, Cartap hydrochloride, Flubendamide (0.005 g a.i) and Carbosulfan (0.1 and 0.2%) 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 in black pepper. The results revealed the efficacy of three isolates (Act 5, Act 2, and Act 9) in reducing the foot rot incidence due to *P. capsici* under challenge inoculation. The isolates also showed 75% increased root development and growth in black pepper rooted cuttings as compared to control. The potential isolates were identified as *Kitasatospora setae* (Act2), *Streptomyces sp.* (Act5) and *S. tauricus* (Act 9).

Development of liquid formulation for Biocontrol agent

Mature conidial suspension in sterile de-ionized 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 *T. harzianum* and *Pochonia chlaydosporia* was also formulated (Fig. 2a & 2b).

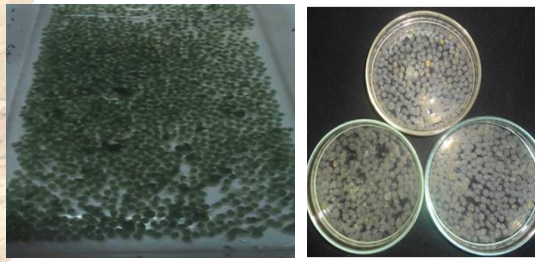


Fig 2a. Encapsulated formulations of Trichoderma harzianum and Pochonia chlamydosporia

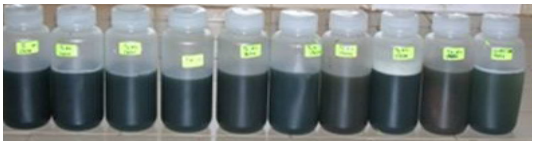


Fig 2b. Liquid formulations of Trichoderma harzianum

Characterization of black pepper *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 1 , Enolase, Heat shock protein 90, TigA gene fusion protein, Mitochondrial genome region between gene Co×2 and gene Co×1 and Ras-related protein (Ypt1) gene. All the loci were amplified using the corresponding primers, which revealed two diverse groups *viz.*, Gp I and Gp II, where Gp I showed similarity to *P. capsici* and Group II showed similarity to both *P. capsici* and *P. tropicalis*.

Targeted re-sequencing and allele mining in *P. colubrinum*

Sequence data analysis of the interactive transcriptome revealed involvement of poly- galacturonases (PGs), pectin lyase and glycosyl hydrolases and other hydrolases of *Phytophthora* in the interactions with *Piper* plants. Sequences with homology to Lectin-like receptor kinase, a potential host target for R × LR type of effectors from *Phytophthora* were also discovered.

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 compared to other defense genes and transcription factors studied (Fig 3).

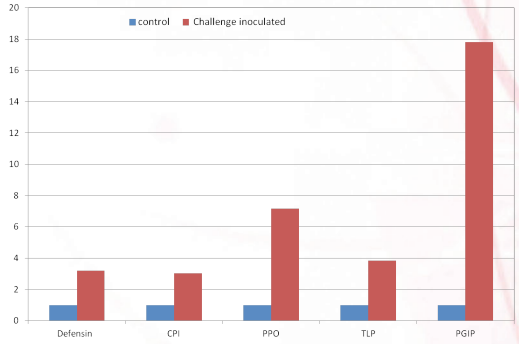


Fig 3. Relative expression of defense related gene in P. colubrinum challenge inoculated with Phytophthora

Evaluation of geographically different isolates of *Trichoderma*

Geographically different isolates of *Trichoderma* when tested against *P. capsici* showed PhytoFuRa10 as highly promising with less than 10% disease incidence followed by PhytoFuRa 8, PhytoFuRa11, and PhytoFuRa13 with 10- 30% disease incidence.

Identification of potential endophytic fungi

Endophytic fungi from black pepper were isolated and evaluated for its biocontrol potential against *P. capsici in vitro*. The *in vitro* inhibition ranged from 70-78% and the isolates were identified based on ITS rDNA sequence as species of *Diaporthe, Phomopsis, Annulohypoxylon nitens, Daldinia eschscholzi*, *Fusarium proliferatum*, *Moniliformae* and *Ceriporia lacerata*.

Radopholus similis diversity

Sixteen isolates of *R. similis* were collected from different parts of Kerala and their genetic diversity was studied 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.

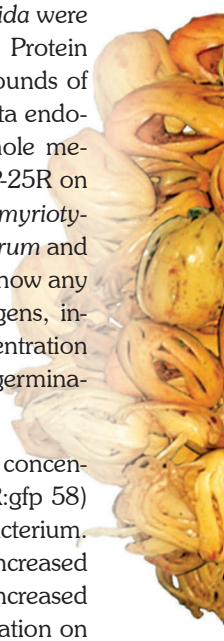
Role of phenyl propanoids in *Radopholus* black pepper interaction

Quantitative assays to estimate phenyl propanoids in black pepper indicated that the levels of anthocayanidins and total phenols were significantly high in HP 39, the nematode resistant black pepper hybrid, compared to the susceptible *Sreekara*. Histochemical studies proved that lignification was significantly high in HP 39 roots, compared to *Sreekara*.

Studies on endophytic bacteria

Endophytic bacteria such as spontaneous rifampicin resistant *Bacillus megaterium* BP-17 R and *Pseudomonas putida* BP-25 R were characterized by Biolog, biochemical and antibiotic sensitivity assays. Reaction of the above two bacteria to 17 antibiotics indicated that *P. putida* BP 25 was resistant to several antibiotics while *B. megaterium* BP 17 was resistant only to rifampicin.

The secondary metabolites of *P. putida* and *B. megaterium* were screened for their activity against fungi, protozoa and oomycetes and for their antihelminthic activity 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 *Phytophthora capsici* while the compounds of *B. megaterium* were docked with 1, 4 beta endoglucanase enzyme of *R. similis*. The 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 20 mg mL^{-1} these extracts inhibited germination of *P. capsici* zoospores.



Colonization studies using different concentrations of gfp tagged *P. putida* (BP-25 R: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}. Bacterial colonization on stem region was noticed only on the 28th day.

Evaluation of fungicides against *Colletotrichum gloeosporioides*

In vitro evaluation of fungicides on growth of *C. gloeosporioides* from necrotic lesion bits obtained from runner shoots showed that the combination product of carbendazim and mancozeb (Fig. 4) was more inhibitory to the growth of the fungus compared to Bordeaux mixture and carbendazim.

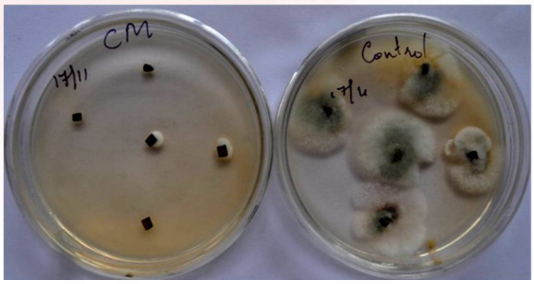


Fig 4. In vitro evaluation of fungicides on the growth of Colletotrichum gloeosporioides

Technology for managing anthracnose disease in nursery

Pre-planting treatment of three node cuttings from the runner shoots with the combination product 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 control of the disease under nursery conditions.

Real-time PCR 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 were developed for sensitive and specific detection and quantification of viruses. Primers were designed from the conserved region to detect all isolates of both the viruses. Standard curve using recombinant plasmid carrying target virus regions of each virus enabled absolute quantification with a wide dynamic range and high sensitivity. 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.

LAMP for detection of viruses

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. The optimum concentrations of magnesium sulphate and betaine besides optimum temperature and time required for successful amplification was determined. 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

CROP IMPROVEMENT

A total of 592 accessions have been maintained in the National Active Germplasm Site for small cardamom 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*, a cardamom variety was registered with Protection of Plant Variety & Farmers Rights Authority New Delhi. Three hundred F2 mapping population from the cross between NKE 12 × GG were artificially inoculated with *katte* virus under green house conditions and 240 were found to be susceptible.

Screening against diseases

Natural incidences of leaf blight and rhizome rot diseases were recorded in 60 accessions maintained at field gene bank. The accessions were further grouped into categories like highly resistant, resistant and moderately resistant based on the reaction towards leaf blight and moderately susceptible, susceptible and highly susceptible against rhizome rot diseases.

CROP PRODUCTION

Drought tolerance studies

Twelve elite lines with three checks were evaluated for drought tolerance under field condition. Moisture stress was imposed by withholding irrigation for two months. Significant variations were recorded for growth and yield characters un-

der stress. Two genotypes with bold capsules and early maturity maintained yield under moisture stress.

CROP PROTECTION

Mycelial compatibility among isolates of *Colletotrichum gloeosporioides*

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

Isolation and characterization of endophytes

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

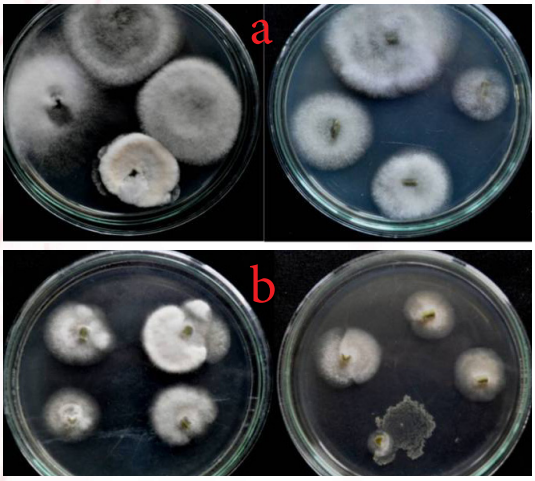
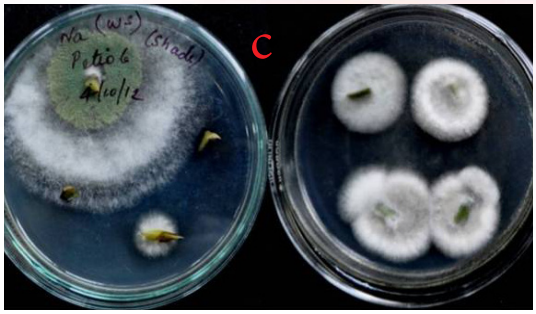


Fig 5. Endophytic fungi and bacteria isolated from cardamom ecotypes (a) Malabar, (b) Mysore and (c) Vazhukka



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 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 *Fusarium oxysporum* were pathogenic to cardamom 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 T 6, RT 7 B and RT 2 A, respectively as the most effective isolates.

qPCR for detection of viruses

A SYBR Green based method for real-time quantitative RT-PCR (qRT-PCR) for detection of Cardamom mosaic virus (CdMV) and Banana bract mosaic virus (BBrMV) infecting cardamom were developed. Standard curve using recombinant plasmid carrying target virus regions of each virus enabled absolute quantification with a wide

dynamic range and high sensitivity. RT-qPCR was more sensitive than conventional RT-PCR in the detection of viruses. The method was validated using field samples collected from different regions.

RT-LAMP for detection of virus

Reverse transcriptase (RT) LAMP method was developed for quick and sensitive detection of BBrMV infecting cardamom. Detection limit of RT-LAMP was higher than conventional RT-PCR. The optimized RT-LAMP was validated by testing field samples of cardamom collected from different regions.

Screening for thrips resistance

Two hundred and ninety six accessions/ lines of cardamom were field screened against the cardamom thrips. Fifty three accessions recorded capsule damage below 20% by thrips. IC 349441 recorded the lowest capsule damage of 3.0%. Five Accessions recorded below 10% capsule damage. Nine accessions recorded more than 70% capsule damage with No. IC 349520 with highest damage of 96.2%. The accessions with less than 10% capsule damage belonged to *Malabar* types except IC 349416 which belonged to *Vazhukka*. All the 9 highly susceptible accessions belonged to either *Mysore* or *Vazhukka* types.

Documentation of bacterial endosymbiont and entomopathogen

The endosymbiont *Wolbachia* was identified in cardamom 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 cardamom thrips

Ten new insecticides and organic products such as neem soap, spinosad, vertemec, thiamethoxam, thiacloprid, imidacloprid, L-cyhalothrin, zolone, fipronil and quinalphos were evaluated in the field for the management of cardamom thrips. Among the treatments, fipronil (1.0 mL L^{-1}) was the most effective in controlling the thrips and was on par with imidacloprid (0.5 mL L^{-1}), quinalphos (2 mL L^{-1}), thiacloprid (0.5 mL L^{-1}), and thiamethoxam (0.3 mL L^{-1}). Neem soap was not effective in controlling the pest population and was at par with control.

TURMERIC

CROP IMPROVEMENT

Genetic resources

One thousand three hundred and forty two *Curcuma* accessions have been planted for multiplication and maintenance. Germplasm conservatory was enriched with 42 turmeric accessions received from Rajendra Agricultural University (RAU), Bihar as part of National active germplasm site (NAGS). Based on request and under material transfer agreement (MTA), germplasm accessions were shared with five research organizations for research. *Curcuma aromatica* (13 accessions), *C. amada* (7 accessions), *C. caesia* (10 accessions) and *C. xanthorrhiza* (15 accessions) were field planted at Peruvannamuzhi. Promising nematode tolerant accessions, Acc. 48 and Acc. 79 have been shortlisted for high yield.

Six novel polymorphic SSR markers (CuMisat 39-44) were isolated by selective hybridization method using 3' biotinylated microsatellite probe mix [(ACT)12, (AAAC)6, (ACCT)6] for identification of different accessions of turmeric (Fig. 6).

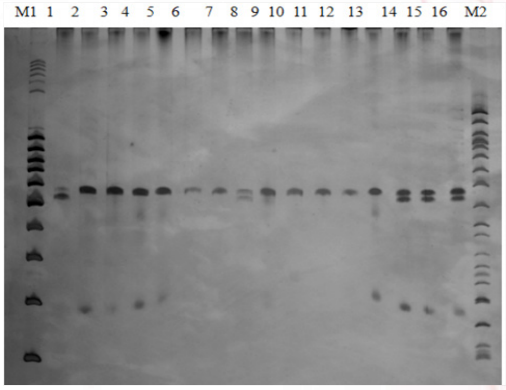


Fig 6. CuMisat 41 showing polymorphism in different accessions of turmeric in 15% PAGE. Lanes 1-16: Turmeric accessions: Alleppey, Amalapuram, Jobedi, Kasthuri, Ayur, Jorhat, Sudarshana, Kedaram, Suguna, Arunachal, Manipur, Dilburgh, Lakadong, Prabha, Pratibha, Suvarna. M1 and M2: 10bp ladder

CROP PRODUCTION

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 turmeric var. IISR *Prathibha* for three years, soil application of zinc up to 10 kg 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 of turmeric, especially in soils deficient in Zn and B (Table 1).

Table 1. Effect of Zn and B on curcumin content (%) of turmeric (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
	P x Zn - *		0.26		L x B - *		0.30

(*sig. at p=0.05)

Organic production package

Organic package consisting of 20 t FYM ha^{-1} + 2 t ha^{-1} neem cake + 1 t ha^{-1} ash + 4 t ha^{-1} vermicompost, *Azospirillum* and P- solubilising bacteria (20 g bed^{-1}) and PGPR (GRB 35) as seed treatment and as drench at 45 and 90 DAP has been standardized. Application of BM (1%) spray to contain foliar diseases and neem oil (5 ml 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 and curcumin content was found to be superior under organic system.

Chemoprofiling of varieties

Essential oil profile of rhizomes of seven varieties of turmeric 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 5-6.8% and Sugandham, *Narendra haldi* and *Co-1* formed another group with 12.5-15.6%.

CROP PROTECTION

Biochemical characterization of resistance for shoot borer

Total fiber and lignin contents in mature shoots; total carbohydrates, lignin and proteins in mature leaves were estimated in both resistant and susceptible accessions. Epicuticular wax, lignin and fiber contents in immature leaf tissues; lignin and fiber contents of immature shoots were determined in both accessions. In mature leaves of resistant accessions; carbohydrates and proteins ranged from 7.558 to 19.709 and 1.079 to 7.754 mg per 100 mg of dried leaves, respectively. The lignin contents ranged from 26.4 to 48.7%. In mature leaves of susceptible accessions, carbohydrates and proteins ranged from 8.779 to 12.761 and 2.822 to 11.104 mg per 100 mg of dried leaves, respectively. The lignin contents ranged from 23.9 to 39.1%.

In mature shoots of resistant accessions, the fibre and lignin contents ranged from 22.6 to 32.7 and 10.6 to 35.6%, respectively. In mature shoots of susceptible accessions, the fiber and lignin contents ranged from 21.2 to 31.8 and 28.1 to 32.8%, respectively. In immature leaves, the epiculicular wax in both susceptible and resistant accessions ranged from 45 to 46 and 44.7 to 45.3 mg per

75 cm^2, fresh leaf. In immature dried leaves of resistant accessions, the lignin and fiber contents ranged from 36.8 to 42.0 and 18.7 to 22%, respectively. In susceptible accessions, the lignin and fiber contents in dried immature leaves varied from 36.5 to 42.0 and 15 to 22%, respectively. The per cent lignin contents in immature shoots of both resistant and susceptible accessions were the same and ranged from 43.0 to 44.0. The fiber contents in immature shoots of resistant and susceptible accessions ranged from 24.3 to 30.3 and 25.7 to 37%, respectively.

Evaluation of EPNs

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

GINGER

CROP IMPROVEMENT

Genetic resources

Six hundred and sixty eight *Zingiber* accessions were planted for multiplication and maintenance. Germplasm conservatory was enriched with 36 collections received from RAU, Dholi, Bihar as part of NAGS. Promising nematode tolerant accession Acc. 219 has been shortlisted for high yield.

Induction of variability through induced mutation

The rhizome buds of three varieties (*Mahima, Varada, Rejatha*) were subjected to gamma irradiation at different doses of 0.5, 0.75, 1.0, 1.25 and 1.50 kR (1kR = 1000 rad) with a check. Based on probit analysis for mortality percent in gamma irradiated ginger, LD50 for three varieties was derived. 5.13% chlorophyll mutation frequency was recorded among M_1V_1 mutants.

Four *Ralstonia solanacearum* resistant mutants (from earlier studies) were planted for multiplication, to take up further yield evaluation. 177 (M_1V6) and 87 (M_1V7) mutants were maintained in pots. One hundred and twenty eight ginger mutants (M_1V7 and M_1V6) were subjected to preliminary screening for soft rot disease caused by *Pythium myriotylum* and 35 mutants which escaped infection were short listed.

NBS-LRR resistance gene analogs in *Curcuma amada*

PCR products amplified using degenerate R-gene primers from the genomic DNA of *C. amada* and *Z.officinale* produced a common band of ~ 500 bp on 1.5% agarose gel. The eluted bands when sequenced resulted in a product size of 531 and 534 nucleotides for *C. amada* and *Z. officinale*, respectively. Cluster analysis of the amino acid sequences of R-genes of *C. amada*, *Z. officinale* and other plant species revealed that the NBS analogs belonged to non-TIR-NBS-LRR subfamily with highly conserved tryptophan (W) as the last residue of kinase-2 domain. Phylogenetic tree constructed using neighbour joining method to visualize the relative distance of the *C. amada* and *Z. officinale* sequences to R-genes and RGCs from other species revealed that *Z. officinale* and *C. amada* belonged to two separate clusters. (Fig. 7 a & b)

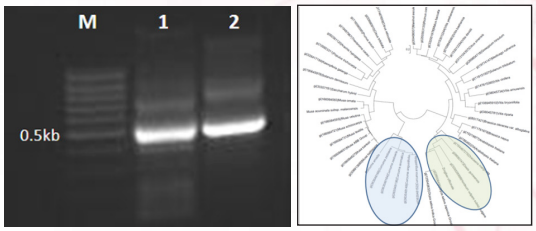


Fig. 7 a) Amplified PCR products of C. amada and Z. officinale . b) Phylogenic relationship of Z. officinale and C. amada RGCs with other plant species

CROP PRODUCTION

Source-sink relationship

Ginger varieties, IISR *Varada*, IISR *Rejatha* and IISR *Mahima* were sampled at monthly intervals starting from 50 days after planting (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 20 t ha^{-1} FYM + 2 t ha^{-1} neem cake + 1 t ha^{-1} ash + 4 t ha^{-1} vermicompost, *Azospirillum* and P solubilising bacteria (20 g/bed) and GRB 35 as seed treatment and as drench and spray at 45 and 90 DAP has been standardized. Drench of PGPR consortia or GRB 35 or GRB 57 and GEB 17 are helpful in keeping the disease incidence to <10-15%. BM (1%) spray to contain foliar diseases and neem oil (5 mL 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 and quality was found to be superior under organic system.

Chemoprofiling of varieties

Volatile oil profile of seven 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%), sesquiphellandrene (11-12%), ar curcumene (8.9-10.3%), bisabolene (1.9-2.6%), and phellandrene (2.8-3.2%). Zingiberene was low in *Rio-de Janeiro* with 16.4% whereas ar-curcumene was low in *Varada* (5.7%).

CROP PROTECTION

PGPR as biocontrol

The rhizobacterial strain GRB 35 confirmed its plant growth promoting efficiency besides reducing soft rot and bacterial wilt in ginger. Field experiment on ginger consisting of treatments involving GRB 35 delivered through various modes *viz.*, Non activated capsule (1 capsule/ 5kg seed)-T1, activated capsule (2 capsule/ 5 kg seed)-T2, activated capsule (1 capsule/ 10 kg seed)-T3, activated capsule (1 capsule/ 5 kg seed)-T4, Talc formulation (10 g/ 10L)-T5. The treatments also included Metalaxyl- Mancozeb (1.25 g/ L)-T6 and an absolute control-T7. The yield data revealed that T2 followed by T1 registered maximum yield (5.38 and 5.35 kg/ $3m^2$, respectively) which was on par with T6 (5.00 kg/ $3m^2$).

Differentially expressed cDNAs in *C. amada* infected by *R. solanacearum*

The functionally annotated supression subtraction hyhidization (SSH) transcriptome revealed defense/stress related genes like Glutathione-s-transferase, leucine rich protein and various enzymes involved in anti-oxidant defense.

Evaluation of antimicrobial properties of *C. amada* against phytopathogens

Essential oil extracted from the dried rhizomes of *C. amada* exhibited maximum antimicrobial activity on *P. myriotylum* and *R. solanacearum*. Beta-Myrcene and beta-pinene were the major components in essential oil.

Phage therapy against *R. solanacearum*

Phages could be 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 (Fig. 8).

Fig 8. Phage plaques on R. solanacearum lawn

Biochemical characterization of resistance for 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 to 13.05 and 1.47 to 4.61 mg per 100 mg of dried leaves, respectively. The lignin contents ranged from 10.05 to 18.94. In mature leaves of susceptible accessions, carbohydrates and proteins ranged from 6.86 to

16. 31 and 1.27 to 2.72 mg 100 mg^{-1} of dried leaves. The lignin contents ranged from 11.13 to 18.08%. In mature shoots of resistant accessions, the fiber and lignin contents ranged from 24.6 to 34.3 and 8.59 to 17.6%, respectively. In mature shoots of susceptible accessions, the fiber and lignin contents ranged from 19.3 to 27.7 and 17.09 to 17.6%, respectively.

In immature leaves, the epiculicular wax in both susceptible and resistant accessions ranged from 20 to 27 and 18 to 27 mg 200 cm^{-2}, fresh leaf. In immature dried leaves of resistant accessions, the lignin and fiber contents ranged from 27.13 to 41.05 and 20-21.5%, respectively. In susceptible accessions, the lignin and fiber contents in dried immature leaves varied from 29.91 to 42.26 and 27 to 28%, respectively. The per cent lignin contents in immature shoots of both resistant and susceptible accessions ranged from 35.43 to 43.03 and 42.56 to 43.47, respectively. The fiber contents in immature shoots of resistant and susceptible accessions were 38 and 30.6%, respectively.

Evaluation of 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 four EPNs namely, *Heterorhabditis sp*. (IISR-EPN 01), *Steinernema sp*. (IISR-EPN 02), *Oscheius sp*. (IISR-EPN 08) and *O. gingeri* in three 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 and six nutmeg accessions were collected. They are *Cheripurathu nutmeg* (entire mace), *Madukkakuzhi* (bisexual, producing more of clustered fruits),

Fig 9. Yellow mace type nutmeg collection

two yellow mace types (Fig. 9), *Kinattukara* and *Kochukudi* (high yielding types).

Garcinia

Biochemical properties of garcinia seed fat of four species were standardized. The butter was solid at room temperature. Melting point varied from 38-40°C and acidity from 3.5 - 3.8. Saponification number varied from 178-200 while it was 184–196 in olive oil, showing its high quality. 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 nine species of garcinia were sequenced and deposited in the NCBI data base (JX472233-JX472241). The evolutionary relationship tree prepared with MEGA5 software produced two distinct clusters. Species from Northeast Himalayan region and from Western Ghats clustered together in the sub cluster showing that the differences in their shape, color and size is due to the environment.

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 8.0% in

ambient condition. 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 cryoground sample compared to 26.0% under ambient condition. Except for β Phellandrene, no major difference was observed in oil constituents.

Total phenol, antioxidant activity on the basis of DPPH assay, phosphomolybdenum method and FRP method using alcohol, water and petroleum ether extracts of variety *Alleppey Supreme* did not show any variation with respect to cryo grinding and other methods of grinding.

Turmeric curing and drying

Experiments on cooking of turmeric at three varying steam pressures (0, 0.5 and 1 kg 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) at Central Institute of Fisheries Technology, (CIFT), Cochin to determine the time required for drying and to evaluate the quality. The cooked samples were dried in CIFT-CRYER-SDL 250 model solar drier from 9.30 am to 4.30 pm. Studies indicated that irrespective of the treatment combinations, the drying time required for bringing down the moisture content to < 10% was seven days.

Experiments on curing of turmeric (var. *Prathibha*) in improved turmeric boiler (TNAU model) and conventional water boiling methods indicated that 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 to the moisture content of < 10%. 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 was reduced to eight days. Quality analysis of the dry turmeric samples indicated reduction in curcumin content from 4.66 % to 4.44% when the curing time increased from 30 min to 90 min in case of improved curing method and from 4.95 % to 4.62 % when the curing time increased from 30 min to 90 min in case of water boiling method.

Production of 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 blend was extruded in a single screw stand alone Brabender extruder at a die temperature ranging from 170 to 190°C at a screw speed ranging from 70 to 90 rpm 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. 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 two 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

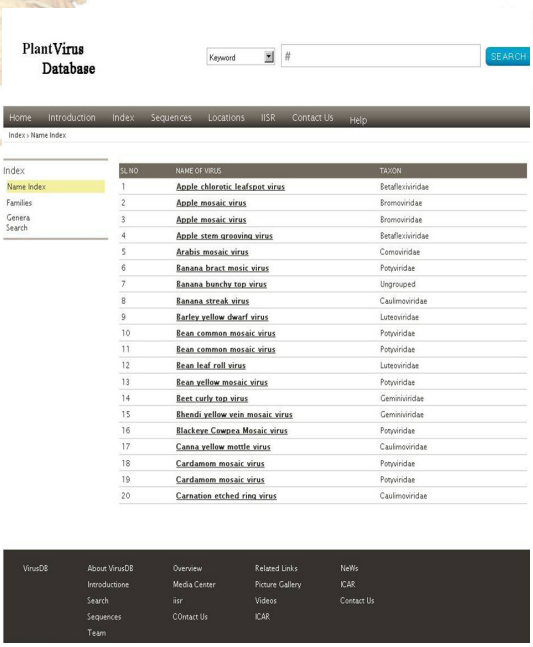
information from multilocus gene trees for four mitochondrial and 10 nuclear markers from six closely related species of Clade 2. The concatenation-based multispecies coalescent approach using Bayesian, maximum parsimony and maximum likelihood methods was able to estimate a moderately supported species 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 (Fig. 10a)
- Phytophthora Genome Database: Sequence information of hybrid assemblies of two isolates of Phytophthora infesting black pepper was incorporated to the existing database (Fig. 10b)

E-procurement module launched

An e-procurement module has been developed and enabled after intensive testing making the procurement of assets more transparent and speedy. Vendor registration and online tender submission have been initiated.



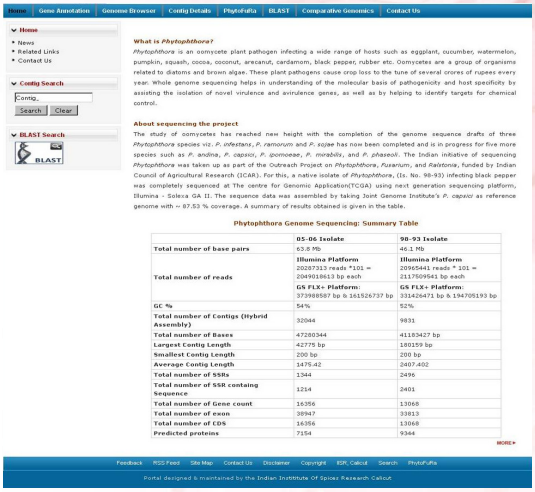


Fig 10. Database on (a) Plant virus and (b) Phytophthora genome

EXTENSION AND TRAINING

Training and capacity building for extension functionaries

An orientation training programme on farm management and spices production technology was offered during 19-21st September 2012 for Technical Assistant recruits of IISR. 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. Under the Horticulture Mission for NE and Himalayan states, 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 18-20th February in which 50 farmers from four districts of the state and eight officers from Department of Horticulture participated. An on demand training programme on black pepper production management was organized from 27-28th February for nine estate managers of Harrison Malayalam Plantations Pvt. Ltd. Also participated in three national and four state level exhibitions for showcasing our technologies.

Impact assessment surveys

The study in Guntur district in Andhra Pradesh revealed that the variety IISR *Prathibha* has spread to about 250 ha as first crop through farmer to farmer lateral spread and exchange of planting material. Even though dominant area is under traditional cultivars like *Dugirala, Kadappa* and *Tekurpet*, the *Prathibha* cultivators reported an average yield of 35 t ha^{-1} whereas the average yield of local cultivars was only around 20 t ha^{-1} (Fig. 11a)

Fig 11a. Prathibha turmeric at Guntur, Andhra Pradesh

Feedback from farmers across different states of India attests the acceptability of *Prathibha* turmeric. In a farm at Vellamunda, Wayanad, Kerala. *Prathibha* was grown in 16 acres (Bucca farms) (Fig 11b).

Fig 11b. Prathibha turmeric at Bucca Farms, Wayanad, Kerala

Studies on Nutmeg showed that IISR variety *Viswashree* has been introduced as a dominant high yielding line as an intercrop in coconut gardens. The buds of established trees were widely used for vegetative propagation in Pollachi region of Tamil Nadu. 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. The popular cropping systems identified are Coconut + Nutmeg, Coconut+ Nutmeg + Arecanut, Coconut + Arecanut + Banana+ Nutmeg and Coconut +Cocoa +Nutmeg.

Soil based nutrient management plans for agro-ecosystems of Kerala

Out of 17069 soil samples from 74 Panchayats of Kozhikode district, 10110 soil samples representing 47 Panchayats have been analyzed for pH, EC, major- and secondary- and micronutrients. 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 was initiated and inputs like planting material, lime, organic manures, neem cake, micro nutrient mixtures and bio agents were supplied (Fig 12).

Fig 12. FLD on management technology of black pepper at farmer's field in Wayanad

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) were broadcast 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 three days. The Farmers Scientist interactive meet was held on 21st December under the chairmanship of Dr. MR Sudarshan, Director (Marketing), Spices Board and was inaugurated by the Hon'ble speaker Karnataka Assembly Mr. KG Bopaiah. Six innovative farmers were felicitated. Various government and self help groups showcased their products/ technologies (Fig. 13).

Fig 13. Inauguration of exhibition by DG, ICAR

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 14).

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 being processed 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 14. License issued to Mr. Tom C Antony, Kottayam for nutmeg variety IISR Viswashree

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. Fourteen Front Line Demonstrations and ten On Farm Trials on technology assessment and refinement were carried out. The Kendra made great impact among farmers, including women by providing training on mechanized coconut palm climbing in collaboration with Coconut Development Board. Most of the trainees are now successful climbers in their localities. Besides, 605 plant-animal clinic consultancy services, 47400 vaccinations of poultry birds and animals and five 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 two radio talks and six study tours for farmers were conducted. During this year Rs.16.69 lakhs has been realized through sale of various technological inputs to farmers. The KVK was also conferred with the best KVK Award 2011 for Zone VIII for its outstanding contributions (Fig 15) which includes a certificate, citation, and cash prize of Rs. 4.0 lakhs.

Fig 15. Best KVK award 2011 for Zone VIII

HUMAN RESOURCE DEVELOPMENT

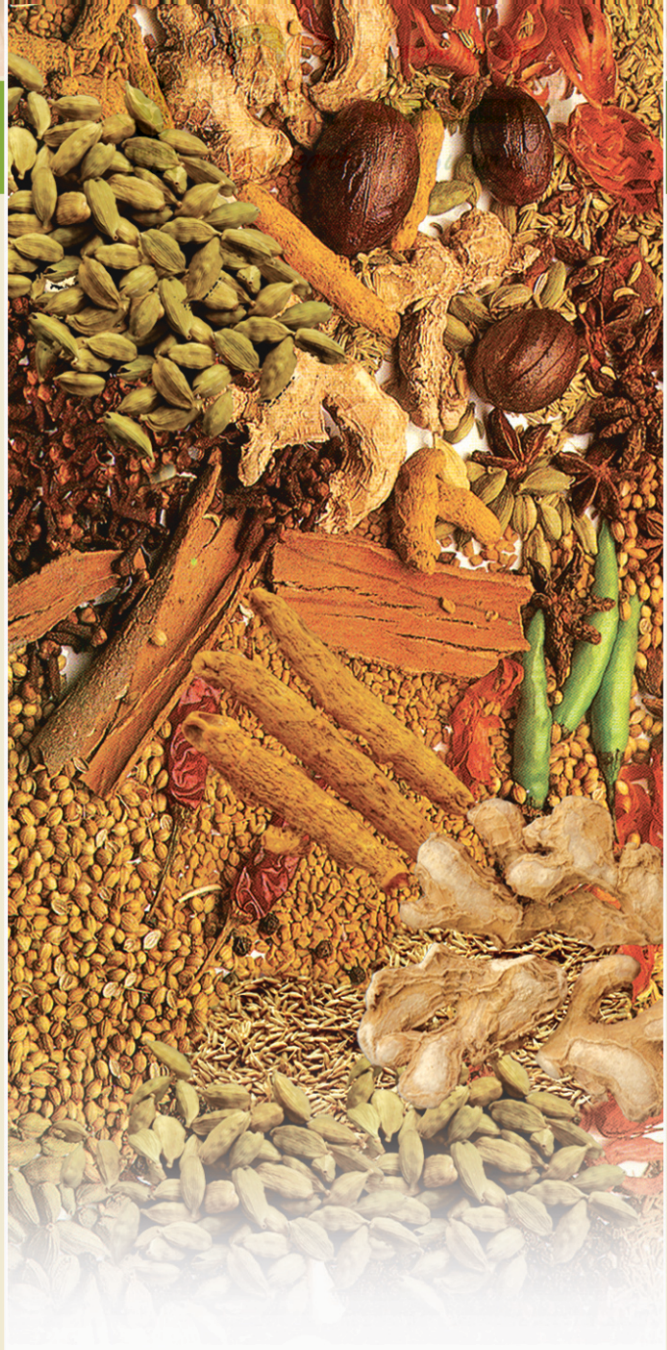
Trainings conducted

Fig 16. Training on Cheminformatics- Tools and Applications

- 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 8th May to 6th June 2012

M.Sc. / Ph.Ds

- One student completed post M.Sc. training and two students were awarded with Ph.D.



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