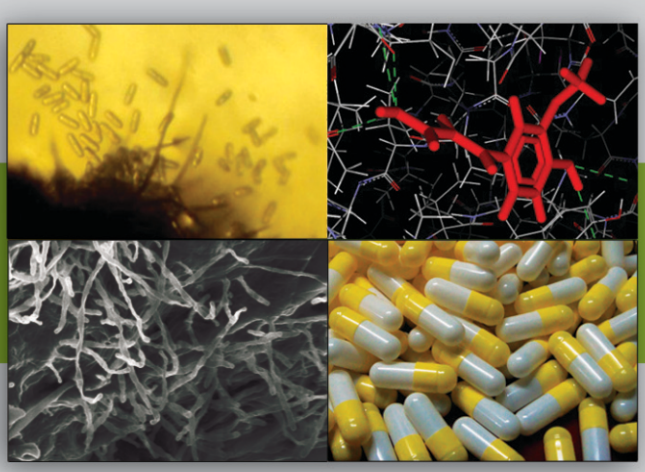
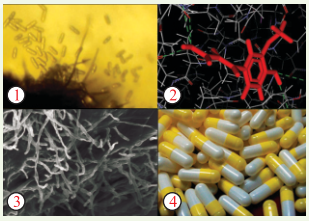
Research Highlights
2013-14



Indian Institute of Spices Research
(Two times winner of Sardar Patel Outstanding ICAR Institution Award)

Kozhikode

Black pepper multiplied in plug trays using soil less nursery mixture



1. Activation of microsclerotia of *Colletotrichum gloeosporioides*
2. Docking pose of ferulic acid with transthyretin (*Radopholus similis*) target
3. Mycelial growth of *Lecanicillium psalliotae* on cardamom thrips
4. PGPR encapsulated in gelatin capsules for delivery to ginger

Research Highlights
2013-14

Indian Institute of Spices Research
(Indian Council of Agricultural Research)
Kozhikode, Kerala, India

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PREFACE

The summary of research achievements of the institute during 2013-14 is presented here as Research Highlights. During this year, collections were made to enrich the cultivar diversity in black pepper from Sirsi, Yellapur, Honnavar and Sagar regions of Karnataka. In nutmeg, notable additions included, a seedless line from Kottayam (Kerala) and 14 monoecious collections from Karnataka. A nutmeg variety 'IISR Keralashree' developed through farmer's participatory breeding has been recommended for release by AICRP on Spices. Two promising lines, one each in cardamom (IC 349651) and turmeric (Acc. 48) with high yield were identified. DNA barcoding was perfected to detect the presence of biological adulterants in traded market samples of black pepper powder.

Results from analyses of cropped soils across all districts of Kerala State indicated the occurrence of acid soils with high levels of phosphorus. Technology for delivering PGPR through capsules for growth promotion and disease control in ginger was developed and validated. A patent for this delivery process has been filed and commercialization is in progress. Healthy planting material production technologies in black pepper (coir pith based medium) and ginger (single sprout transplanting) were standardized.

Technologies for management of anthracnose disease in black pepper and leaf blight in cardamom were developed. Evaluation of chemicals against foot rot (*Phytophthora capsici*), and burrowing nematode (*Radopholus similis*) of black pepper and thrips of cardamom (*Sciothrips cardamomi*) has given good leads. Testing of efficient *Trichoderma* strains against *P. capsici* in black pepper revealed that some of the isolates were efficient in disease suppression irrespective of the location or host plant indicating the adaptation of isolates to various niches. Complete genome sequencing of *Piper yellow mottle virus* (PYMoV) and genetic diversity analyses revealed occurrence of PYMoV in eight *Piper* spp. and additional new distinct badna viruses in four *Piper* spp. We also recorded the occurrence of the entomopathogenic fungus (*Lecanicillium psalliotae*) isolated from cadavers of cardamom thrips, which is also the first report of a fungus infecting cardamom thrips. Perennation of *Colletotrichum gloeosporioides* in the perfect stage was recorded in black pepper that has epidemiological significance.

Infrastructure for healthy planting material production of spices was created. The institute participated in 10 off campus exhibitions/Farmers melas. A Technology Week was celebrated at KVK, Peruvannamuzhi during January 2014. About 151 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted and 5139 trainees were benefitted. Eleven front line demonstrations and six on farm trials on technology assessment and refinement were carried out.

I consider it a privilege to place on record the encouragement given by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR. We are also grateful for the strong support and guidance received from Dr. N.K. Krishnakumar, Deputy Director General (Horticulture) and Dr S.K. Malhothra, ADG (Hort. II). I appreciate the efforts and zeal shown by all the project investigators in executing various programmes. The financial support for the projects received from ICAR is gratefully acknowledged. I also commend the editors for having compiled and brought out this publication.

M. Anandaraj
Director

Kozhikode
15.03.2014

CONTENTS

BLACK PEPPER

Genetic resources

Wild relatives and cultivars of black pepper were collected from Sirsi, Yellapur and Honnavar Taluks of Uttar Kannada and Sagar regions of Shimoga district. Twenty one accessions of cultivars and five wild relatives were collected during this exploration. The present status of black pepper germplasm accessions conserved at the gene bank is 3181 (Wild pepper- 1503, Cultivars- 1669, Exotic species- 9). Out of 2342 germplasm accessions screened for resistance against *Piper yellow mottle virus*, four accessions showed resistance in the preliminary test.

A germplasm block consisting of 142 accessions was established at Central Horticultural Experiment Station (CHES), Chettalli as an alternate center. Improved varieties and examples varieties (DUS) were planted for conservation as well as top shoot production under protected conditions. IC numbers were obtained for 224 accessions of black pepper.

Breeding

Hybridization was undertaken using Subhakara as female parent and bold berried accessions *viz.*, Vadakkan and Waynadan bolt as male parents for development of lines with bold berries. Attempts were also made to hybridize with one of the wild accessions of *Piper galeatum*.

Piper nigrum - *Phytophthora* interaction

Isolation of resistance gene candidates

PCR amplification with R-gene-specific degenerate oligonucleotide primers resulted in amplification of 500bp product in IISR Shakthi, Sreekara, Subhakara, P24-O-4, *Piper colubrinum* (Acc. 392) and *P. ornatum* (Acc. 3362), which was then sequenced. The similarity of these sequences to other *Piper* RGAs ranged from 40% to 51% and 78% to 99%. BLASTP searches of deduced amino acid sequences revealed the presence of NB-ARC (nucleotide-binding and similarity to Apaf-1, R genes and ced-4) domain. Further analysis of the sequences using ORF finder revealed that 39 out of 51 could be translated into a single open reading frame (ORF) of considerable length of more than or equal to 100 amino acids. Multiple alignments of amino acid sequences revealed the presence of kinase2a internal to PLOOP and GLPL motifs. In addition, the analysis showed a tryptophan (W) residue at the end of kinase-2 motif, a characteristic feature of non-TIR subclass of NBS-LRR R genes.

Proteogenomics

2D proteomics coupled with mass spectrometry yielded many black pepper proteins. The identified proteins provide functional information in this crop and also ensure an excellent experimental procedure for studying black pepper–*Phytophthora* interactions.

Targeted expression analysis of resistance genes

The R genes (NBS4 and NBS5) expression pattern by qPCR was different between resistant (IISR Shakthi) and susceptible (Subhakara) lines suggesting that R genes have a distinct pattern of expression and play a critical role in *Phythophthora capsici* (05-06) stress tolerance. There was an observed expression shift of R genes at various times after inoculation. The resistant cultivar showed early response when compared to the susceptible one.

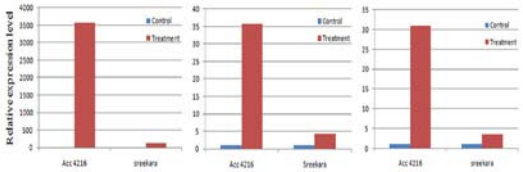
Expression analysis of putative R genes

Real time PCR analysis using cDNA prepared from *Phytophthora* inoculated and uninoculated leaves revealed the expression level of the three putative R genes (LR 2277, LR 1990 and PCR 07) at different hours of post inoculation with *P. capsici* 05-06 strain and 98-93 strains. Highest level of expression was noticed in case of LR 1990 when challenged with 05-06 while LR 2277 gene expressed maximum with the isolate 98-93. Maximum expression of the putative R gene, LR 2277

was observed in the initial period of pathogen interaction and there was a decrease in expression with time, whereas the expression of the other two genes *viz.*, LR 1990 and PCR 07 was maximum at 16 hpi.

Expression analysis of water deficit stress-induced genes

The expression level of water deficit stress-induced genes *viz.*, dehydrin, osmotin and a regulatory protein, dehydration responsive element binding (DREB) of black pepper was studied using qPCR. The genes showed significantly higher expression in tolerant variety under stress, the maximum expression being observed in case of dehydrin (Fig. 1). The expression analysis of three genes suggested that drought tolerance in black pepper is associated with a rapid modulation of genes from different gene families.



(a) (b) (c)

Fig. 1. Expression pattern of different genes (a) dehydrin (b) osmotin and (c) DREB protein in response to water deficit stress in Acc. 4216 and Sreekara. The relative expression was calculated using Actin as an internal reference. The unstressed expression level was assigned a value of 1.

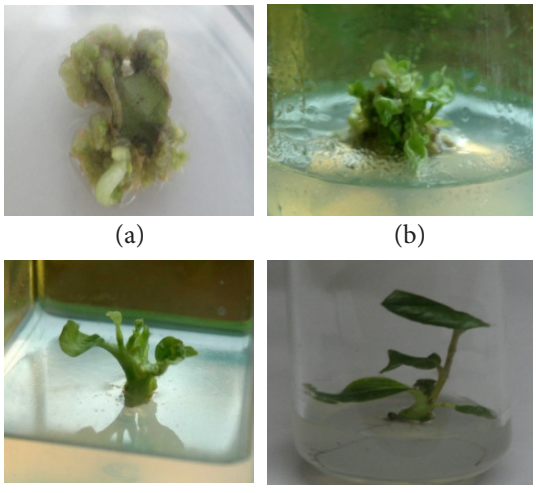
Tissue culture

Meristem culture technique using liquid culture medium for the production of plantlets from 2.0 mm shoot tips was standardized (Fig. 2).

Fig. 2. Black pepper plantlets through meristem culture

Direct shoot organogenesis

A regeneration protocol *via* direct shoot bud formation was standardized using greenhouse grown leaf explants of *Piper colubrinum*. Maximum number of shoots was produced from leaf discs cultured on half strength MS medium II supplemented with 2mg L^{-1} BA and 0.01mg L^{-1} NAA. Plant regeneration and rooting of the plants took four months from culture initiation (Fig. 3).



(a) (b) (c) (d)

Fig. 3. *In vitro* shoot bud formation and plantlet regeneration from leaf explants of *P. colubrinum* (a) shoot bud induction at the cut end of leaf (b) multiple shoot elongation (c) single shoot transferred to rooting medium (d) rooted plantlet

Soil carbon sequestration

Soil samples were collected (0-25 cm) from high density cropping systems having coconut, banana, nutmeg, cinnamon and black pepper component crops at Central Plantation Crops Research Institute (CPCRI), Kasaragod and the C build up in terms of total organic carbon (TOC) and particulate organic carbon (POC) at the basin of different component crops was studied. The POC and TOC contents were found to be higher under black pepper (11.6 g kg^{-1} and 35.2 g kg^{-1}) followed by nutmeg and coconut. The total organic nitrogen content also was found to be higher under black pepper followed by coconut. POC constituted 18-33% of the TOC content.

Acidity and P toxicity in soils

Results from analyses of cropped soils across all districts of Kerala State indicated the occurrence of acid soils with high levels of phosphorus (P). About 91% of the soil samples tested was acidic, with 54% of the samples testing for strong to extremely acid reaction (Fig. 4), while 62% of the samples registered high (25-35 kg ha^{-1}) to extremely high (100 kg ha^{-1}) available P levels (Fig. 5).

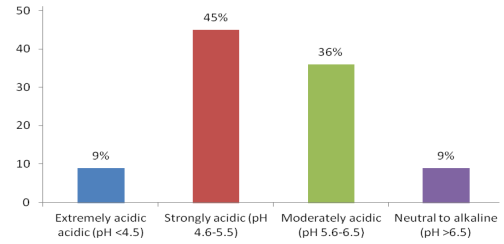


Fig. 4. Frequency of soil pH classes across all districts of Kerala state (n= 156801)

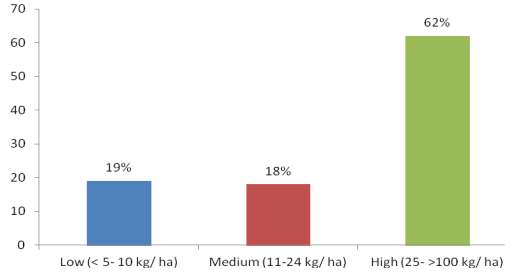


Fig. 5. Frequency of soil available phosphorus classes across all districts of Kerala state (n= 150281)

Propagation

Among the different nursery media combinations studied for black pepper multiplication using soil-less nursery mixture, coir pith with *Trichoderma* and vermicompost in plug trays recorded significantly higher nursery growth parameters than all other treatments. Among the single node cuttings with three different maturities (collected from the serpentine method runners), maximum growth in nursery was recorded in the terminal portion of the runners (11-15th nodes). Similarly, higher nursery growth parameters were recorded in the cuttings planted with full leaf compared to half-leaf (Fig. 6).

Fig. 6. Growth of black pepper cuttings in plug-trays

Post harvest technology

Quality profile of cryogenically ground black pepper

Black pepper (Panniyur-1) powdered using cryogenic grinder at 10°C and at -50°C at varying screw speeds indicated that moisture retention was 14% at -50°C compared to 11% at 10°C. Essential oil recovery was 2% at -50°C while at 10°C it was 1.6%. Piperine, total phenol and antioxidant activity in terms of di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) activity did not vary with respect to temperature and screw speed. Retention of essential oil constituents like α, β pinene, limonene, and β-caryophyllene content was high at -50°C compared to grinding at 10°C.

Production of white pepper

Experiments on production of white pepper from freshly harvested green pepper indicated that enzyme production was maximum on the fifth day in the fermentation medium when *Bacillus subtilis* (MTCC 5406) was used with enzyme activity of 120.5 Unit mL^{-1} and complete decortication of outer skin was obtained on the 6th day when cleaned and washed manually. Decortication was also completed on the 6th day with *B. subtilis* (MTCC 5407). Under similar temperature, *B. licheniformis* (MTCC 5408) recorded the lowest enzyme activity (52.83 Unit mL^{-1}) at 48 h.

Barcoding of adulterants

DNA barcoding, using the *loci rbcL, matK, rpoC1* and *psbA-trnH*, was perfected to detect the presence of biological adulterants in traded market samples of black pepper. Two out of nine market samples tested were found to be adulterated with chilli. The locus *psbA-trnH* proved to be best for adulteration detection as band level detection with this locus yielded a band of 350 bp and chilli yielded one of 600 bp size (Fig. 7). The market samples were, however, free of *P. galeatum* and *P. attenuatum* (wild species) adulteration. *rbcL* and *rpoC1* could differentiate *P. attenuatum* from *P. nigrum* and *P. galeatum* while *psbA-trnH* differentiated *P. galeatum* from *P. nigrum* and *P. attenuatum*. Adulteration even at very low levels (0.5%) could be detected using barcoding locus.

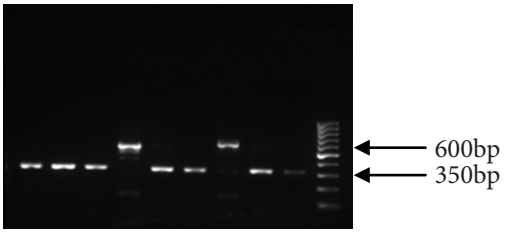


Fig 7. Amplification of *psbA-trnH* locus (lane 1- *P. nigrum* (Acc. 6834), lane 2- *P. nigrum* (Acc. 6857), lane 3- *P. nigrum* (Acc. 6833), lane 4- *Capsicum annum*, lane 5-MS- 1, lane 6-MS-2, lane 7-MS-3, lane 8- MS-4, lane 9-MS-5, lane10-100bp ladder)

Phytophthora foot rot and slow decline

Collection and maintenance of *Phytophthora* isolates

In a survey conducted in Idukki, Kasaragod and Wayanad districts of Kerala, 55 new isolates of *Phytophthora* were collected from different hosts and added to the National Repository of *Phytophthora*; the repository presently has 442 isolates.

Screening open pollinated progenies

Thirty eight open pollinated progenies of IISR Shakthi along with the parent and Subhakara (check) were screened for *Phytophthora* resistance by leaf and stem inoculations. Among the 38 progenies screened, IISR Shakthi OP 116 was the most susceptible; IISR Shakthi OP 103 took up leaf infection but tolerated stem inoculation with an average of 4 mm lesion length after 72h of inoculation.

Genome sequencing and annotation

Whole genome alignment of *P. capsici* and comparison with the reference genome (JGI) revealed SNP sites, common genes and genes unique to *P. capsici* isolate of IISR. Blast homology based functional annotation revealed the presence of various proteins important for the survival of *Phytophthora* sp. in host plants and virulence associated proteins crucial for its infection. Pairwise comparison synteny plot of gene models, *P. capsici* whole genome of JGI to *P. capsici* of IISR (05-06) with PROmer package of MUMmer was completed. The SNPs were integrated and mapped with the whole genome sequence data.

Disease management

Evaluation of new chemicals against *Phytophthora capsici*

Two new strobilurin fungicides *viz*., Ergon 44.3% (w/w) [Kresoxim methyl 500 gL^{-1} SC] and RIL-070/FI (72WP) were evaluated *in planta* against *P. capsici*. Ergon was evaluated at 5000-8000 ppm concentrations. Maximum inhibition (57.1%) was observed five days after spray at 7000 ppm. Soil application at different concentrations (6000-8000 ppm) showed no infection or mortality of plants. RIL-070/FI, when evaluated in planta at 100-600 ppm concentrations showed 100% inhibition of lesion development when *P. capsici* was challenge inoculated five days after spray at 600 ppm. However, soil application of the chemical at 400 ppm showed 100% disease suppression and *P. capsici* population was reduced by 77.6%.

Evaluation of consortia of actinomycetes

Four promising actinomycetes (Act 2, Act 5, Act 6 and Act 9) were evaluated individually and in consortia mode under greenhouse conditions for growth promotion and disease suppression. Growth promotion was promising in consortia containing Act 2 + 5, Act 2 + 9 and Act 5 + 9 (Fig. 8).

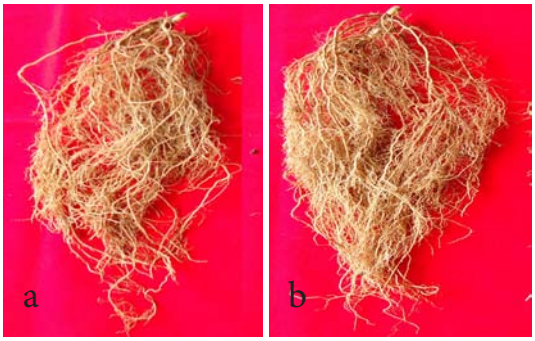


Fig. 8. Root development in promising consortia of actinomycetes (a) Act 2 + 5 and (b) Act 2 + 9

Evaluation of *Trichoderma* isolates

Trichoderma isolates obtained from various geographical locations were evaluated against *P. capsici* under pot culture conditions for growth promotion and disease suppression. Among the 15 isolates, PhytoFuRa10 was highly promising (82.96% disease control), followed by PhytoFuRa 8 and PhytoFuRa15 (65.5% and 63.38% disease control, respectively) when compared to control (85.6% disease incidence) where no *Trichoderma* was inoculated.

Evaluation of chemicals against *Radopholus similis*

Nematicidal activity of five chemicals *viz.*, fipronil (10 and 15 g pot^{-1}), thiamethoxam (0.5 and 1 g pot^{-1}), carbosulfan (G) 5 and 10 g pot^{-1} and carbosulfan (0.1% and 0.2%) were evaluated against *R. similis* under pot culture conditions among which fipronil (15 g pot^{-1}) and carbosulfan 0.1% were found to be promising.

Development of liquid formulation for *Pochonia chlamydosporia*

The survival of *P. chlamydosporia* in different liquid formulations was evaluated for studying the shelf life of the organism in liquid media. Eleven different formulations *viz.*, glycerol 10 and 25%, glucose 10, 25 and 50%, DMSO 5, 10 and 25% and liquid paraffin 5, 10 and 25%, were tested among which liquid paraffin (5%) could maintain effective population (cfu) of the biocontrol agent for 120 days.

Role of phenyl propanoids in *Radopholus*-black pepper interaction

A new set of compounds in phenyl propanoid metabolic pathway of black pepper were screened for potential target inhibiting activity using eight targets in *R. similis* and the mechanism was studied based on molecular docking. The study revealed that 13 phenylpropanoids had very low dockscores and possessed more number of hydrogen bonds than the available nematicide, carbofuran. The study also showed that carbofuran and phenylpropanoids were interacting highly with three potential targets *viz.,* calreticulin1, GST and a transthyretin-like protein. Screening of these compounds under *in vitro* conditions showed that eight among the 13 phenylpropanoids (syringaldehyde, salicylic acid, catechol, ferulic acid, coumaric acid, caffeic acid, tannic acid and N-vanillylnonanamide) caused maximum mortality of *R. similis* at 200 ppm.

Studies on endophytic bacteria

Colonization of *Pseudomonas putida* induced the activity of defense enzymes like peroxidase by 25.0% and 49.4% at 48 h in roots and leaves, respectively, while the increase was 38.5% and 37.7%, respectively for phenyl ammonia lyase; polyphenol oxidase showed higher activity in bacteria colonized plants at 96 h. *In vitro* bioassays with phenazine, a secondary metabolite from *P. putida*, inhibited mycelial growth of *P. capsici* at ≥

60 ppm. The minimum inhibitory concentration (MIC) of phenazine causing 50% of inhibition of *P. capsici* on 1/5th PDA was 0.02 mg mL^{-1} and MIC causing total inhibition was 0.06 mg mL^{-1}.

Piper yellow mottle virus (PYMoV)

Complete genome sequencing

Complete genome sequencing of PYMoV from black pepper, betelvine and Indian long pepper was performed to understand the genetic variability of the virus in different hosts. The genome length varied from 7549 to 7607 nucleotides in different hosts and all the three genomes possessed four open reading frames (ORF). Whole genome sequence comparison showed an identity of 89%- 99% with one available PYMoV sequence while it ranged from 39%-56% with other badnavirus species indicating that badnavirus infecting black pepper, betelvine and Indian long pepper are strains of PYMoV. In phylogenetic analysis, PYMoV sequences were clustered together with two subgroups: PYMoV from black pepper grouped in one subgroup while PYMoV from betelvine and long pepper in another subgroup. Other badnaviruses found closely related to PYMoV included *Dioscorea bacilliform virus, Fig badnavirus 1, Cacao swollen shoot virus* and *Citrus yellow mosaic virus.*

Genetic diversity

The conserved reverse transcriptase (RT) / ribonuclease H (RNase H) coding region of the virus was cloned and sequenced from 13 PYMoV isolates of black pepper collected from different cultivars and regions and one isolate each from 23 other species of *Piper* to understand the genetic variability of the virus. All isolates from *P. argyrophyllum, P. attenuatum, P. barberi, P. betle, P. colubrinum, P. galeatum, P. longum, P. ornatum, P. sarmentosum* and *P. trichostachyon* showed an identity of >85% at the nucleotide and >90% at the amino acid level indicating that they are strains of PYMoV. On the other hand high sequence variability (21%-43% at nucleotide and 17%-46% at amino acid level compared to PYMoV) was found among isolates infecting *P. bababudani, P. chaba, P. peepuloides, P. mullesua* and *P. thomsonii* suggesting that they may represent the genome of new badnaviruses. Phylogenetic analyses showed close clustering of all PYMoV isolates that were well separated from other known distinct badnaviruses. This is the first report of occurrence of PYMoV in eight *Piper* spp. and additional new distinct badnaviruses in four *Piper* spp.

Influence of temperature on symptom expression

Symptomless PCR positive and negative plants of black pepper cuttings were exposed to 35°C, 60% RH for 8 h daily. In PCR positive plants, typical virus symptoms started appearing on 10th day indicating that temperature has direct influence on symptom expression. Symptomatic plants had higher content of total proteins, IAA and reducing sugars. Analysis of total proteins extracted from leaves of PCR positive and negative plants before, during and after exposure to temperature through 2-D electrophoresis coupled with mass spectrometry analysis yielded major host proteins which will have influence on symptom expression.

Anthracnose

Epidemiology

Studies on activation of microsclerotia of *Colletotrichum gloeosporioides* in runner shoots of black pepper showed that the microsclerotia were activated within 7 days when subjected to high humid conditions by producing acervuli with setae (Fig. 9 a, b, c) and subsequent production of conidia embedded in a matrix (Fig. 9. d, e) under *in vitro* conditions.

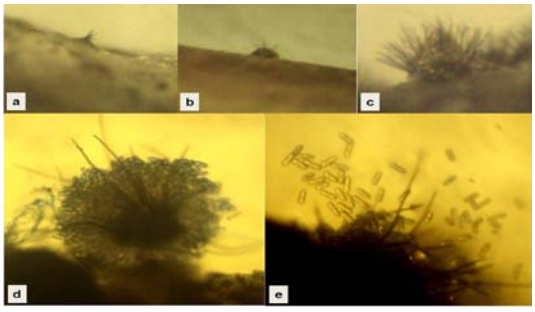


Fig. 9. Activation of microsclerotia of *C. gloeosporioides* (a-e)

Surveys revealed the incidence of foliar infection characterized with grey necrotic lesions with black borders on older leaves of preceding season and unevenly distributed minute dark structures on the foliage of nursery plants. The dark structures produced orange coloured exudation, when incubated under high humid conditions. Microscopic observations revealed the presence of asci, ascospores and perithecia embedded in the exudate. The leaf bit with exudate when inoculated on the black pepper variety, Panniyur-1, resulted in the formation of typical anthracnose symptoms. Subsequent isolation from the lesion yielded two distinct colonies, designated as black and orange. Pathogenictiy of the cultures was proved on Panniyur-1 by foliar inoculation of the cultures separately and in combination, which resulted in the manifestation of symptoms within three days after inoculation.

Field validation of management strategies

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb, carbendazim, Bordeaux mixture and hexaconazole, and soil application of *T. harzianum*, singly and in combination showed that spraying carbendazim + mancozeb 0.1% thrice at 30 days intervals was superior over other treatments in reducing anthracnose incidence.

CARDAMOM

Genetic resources and breeding

A total of 618 accessions have been maintained in the National Active Germplasm Site at Cardamom Research Centre (CRC), Appangala. Sixty accessions were characterized for yield and yield contributing characters. Natural incidence of leaf blight (*Colletotrichum gloesporoides*) and rhizome rot disease was recorded in 60 accessions maintained in the field gene bank at Appangala. Thirty two and 14 accessions were found resistant to leaf blight and rhizome rot, respectively.

IC 349651, a high yielding cardamom line has been shortlisted for release with average yield of 1030 kg ha^{-1}. Twenty one inter-varietal F1 hybrids are shifted to main field for yield studies. Also, 23 selfed progenies have been shifted to the main field for thrips tolerance studies.

Standardizing the parameters for targeted yield

Based on the previous year's crop yield under different treatments and the nutrient uptake data, the nutrient removal for producing 100 kg of capsule was worked out for Appangala-1 and Green Gold varieties. The nutrient contribution from soil was 34.1% for N, 4.3% for P_2O_5 and 14.8% for K_2O (Green Gold) and 17.3% for N, 7.3% for P_2O_5 and 8.3% for K_2O (Appangala-1). The nutrient contribution from fertilizer was worked out to be 26.6% for N, 4.35% for P_2O_5 and 15.2% for K_2O (Green Gold) and 11.4% for N, 2.7% for P_2O_5 and 7.1% for K_2O (Appangala-1). Spraying of micro nutrient mixture, IISR Power Mix twice at 5g L^{-1} during June and August resulted in 10.3% increased capsule yield as compared to control.

Leaf blight

Validation of management strategies in the nursery

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb, carbendazim and Bordeaux mixture, and soil application of *T. harzianum*, singly and in combination showed that, spraying carbendazim + mancozeb (0.1%) at 30 day intervals was promising in reducing leaf spot incidence.

Validation of management strategies in the field

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb, carbendazim and hexaconazole, and soil application of *T. harzianum*, singly and in combination showed that combined application of hexaconazole 0.1% and soil applica-

tion of *T. harzianum* thrice at 30 days interval was promising in reducing leaf blight incidence.

Rhizome–root rot

Identification of primary causal organism

Inoculation studies under glasshouse conditions with *Pythium vexans, Rhizoctonia solani* and *Fusarium oxysporum* individually and in combination on cardamom seedlings (var. Appangala-1) indicated that inoculation with *P. vexans* alone resulted in 66.7 % mortality whereas, sequential inoculation of *P. vexans* followed by *R. solani* recorded 83.3% mortality.

Colonization and proliferation of pathogens

Studies on colonization and proliferation of *P. vexans, R. solani* and *F. oxysporum* showed that *P. vexans* required 4 h to colonize the roots, whereas *R. solani* and *F. oxysporum* required 12 and 96 h, respectively. Under high humid conditions, sporangia of *P. vexans* were produced in abundance and aggregated near the root tip region. The sporangia germinated either directly by germ tubes (Fig. 10 a) or indirectly, through the formation of vesicles containing zoospores (Fig. 10 b). *R. solani* initially produced primary and secondary hyphal branches and several side branches formed were later modified into infection structures like bulbous and lobate appressoria.

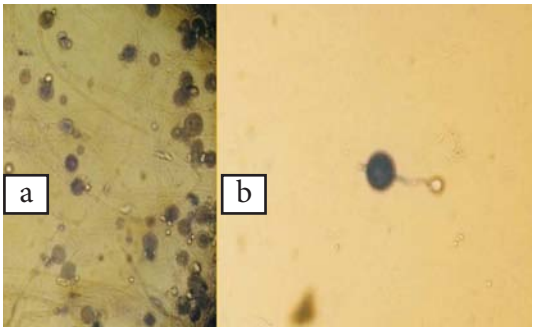


Fig. 10. Germination of sporangia (a) directly by germ tubes or (b) indirectly, through formation of vesicles containing zoospores

In vitro screening of antagonists

Under *in vitro* conditions, nine isolates of *Trichoderma viz.*, KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were effective against *P. vexans, R. solani* and *F. oxysporum*.

In vitro screening of chemicals

Among the seven fungicides tested against *P. vexans*, fenamidone + mancozeb 0.2% and captan + hexaconazole 0.2% were effective under *in vitro* conditions. Fenamidone + mancozeb 0.2% and tebuconazole 0.05% were effective against *R. solani* whereas, tebuconazole 0.05% was superior over other fungicides against *F. oxysporum*.

Isolation of endophytes

Isolations made during the monsoon period from leaves, petioles, pseudostem, roots and rhizomes of *Amomum microstephanum, Alpinia mutica, Alpinia galanga* (two collections), *Amomum subulatum, Aframomum melegeuta, Amomum sp., Hedychium coronarium and Zingiber zerumbet* yielded 82 fungal and 10 bacterial isolates. Four fungi were isolated from surface sterilized samples of capsules and seeds of Mysore ecotype. Among the isolates, III B (isolated from capsule) was found to have inhibitory effect on the growth of *C. gloeosporioides*.

Cardamom thrips

Screening of cardamom lines for resistance

Field screening of cardamom germplasm at CRC, Appangala for identification of sources of resistance to thrips continued for the third consecutive year in association with Indian Institute of Horticultural Research (IIHR), Bengaluru. IC 349455 recorded the lowest total capsule damage of 8.3%, followed by IC 547144 (10.2%). These two accessions belonged to *Malabar* type. Sixteen accessions recorded more than 80% total capsule damage. IC 349582 showed highest damage of

98.5% followed by IC 349540 (94.4%). Both these accessions belonged to *Vazhukka* type.

Evaluation of insecticides and natural products

Eleven insecticides and natural products *viz.*, neem soap, spinosad, abamectin, thiamethoxam, thiacloprid, imidacloprid, L-cyhalothrin, phosalone, fipronil, dinotefuron and quinalphos were evaluated in the field at CRC, Appangala, for the management of cardamom thrips in association with IIHR, Bengaluru. Five sprays of the test products were sprayed during March, April, May, August and September. The trial indicated that among the treatments, fipronil (1.0 mL L^{-1}), quinalphos (2 mL L^{-1}) and imidacloprid (0.5 mL L^{-1}) were effective and on par with each other in controlling the thrips population. Combined analysis for three years indicated that fipronil (1.0 mL L^{-1}), imidacloprid (0.5 mL L^{-1}) and thiamethoxam (0.3 mL L^{-1}), were more effective and on par in controlling the pest.

Studies on bacterial endosymbionts

The status of infection of the bacterial endosymbiont *Wolbachia* in thrips population varied from 15.0%-87.8 % in various areas of Kerala, Karnataka and Tamil Nadu. The mean infection rate was 53.5% with 57.1% male and 50.6% female population. The sequence data generated for the wsp surface protein using wsp specific primers and the primers specific to super group B and *Con* sub-group were deposited in NCBI GenBank. Phylogenetic analysis revealed that all the *Wolbachia* isolates from cardamom thrips collected from different areas clustered together showing 99% similarity indicating that irrespective of geographical isolation, all the thrips were infected by the same *Wolbachia* strain, wScar.

Studies on entomopathogens

An entomopathogenic fungus isolated from cadavers of cardamom thrips from Wayanad district was identified as *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales). Laboratory bio-assays with purified conidial suspension of the fungus confirmed the infectivity of the fungus to cardamom thrips. At the highest dose tested (1×10^7 conidia mL^{-1}), up to 62.9% mortality was recorded in the test population, 10 days post inoculation. The ITS rDNA, partial ß-tubulin and partial translation elongation factor 1α genes of this fungus was sequenced and the sequence data submitted to NCBI GenBank. This is the first record of occurrence of *L. psalliotae* in India and also the first report of a fungus infecting cardamom thrips.

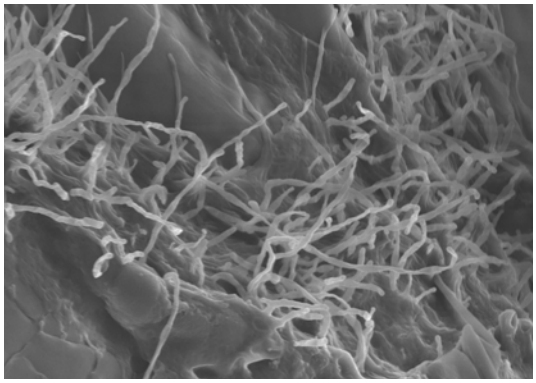


Fig. 11. Mycelial growth of *Lecanicillium psalliotae* on cardamom thrips

A technology for mass multiplication of *L. psalliotae* for field application was standardized. Soaked and half boiled paddy grains were found to be suitable for large scale multiplication of the fungus.

Documentation of natural enemies

Surveys were conducted in over 75 locations in nine districts of Kerala, Karnataka and Tamil Nadu to document entomopathogens and other natural enemies of insect pests of spice crops. Eight entomopathogenic fungi belonging to *Isaria* sp., *Paecilomyces* sp. and *Lecanicillium* spp. were isolated from scale insects infesting black pepper (*Lepidosaphes* sp., *Marsipococcus* sp. and *Protopuluvinaria* sp.) and cardamom (*Aulacaspis* sp.). Three larval and three pupal parasitoids belonging

to Braconidae, Ichneumonidae and Tachinidae were recorded in shoot borer infesting ginger and cardamom. Coleopteran predators such as *Chilocorus circumdatus* and *C. nigritus* were recorded on mussel scale infesting black pepper.

GINGER

Genetic Resources

Six hundred and sixty eight *Zingiber* accessions have been maintained in the field gene bank. Germplasm conservatory was enriched with a extra bold local accession from Arunchal Pradesh.

Breeding

Evaluation of extra bold and low fibre ginger accession led to the identification of three accessions (Acc. 726, Acc. 91 and Acc. 247) with high yield and bold rhizomes.

Fig. 12. An extra bold ginger accession (Acc. 726)

Four thousand one hundred and twenty ginger rhizome buds were subjected to gamma irradiation at different doses (0.80, 0.90 and 1.00 kr). The M1V1 mutants are established in the green house for screening against *Pythium* sp. Screening of 300 M1V2 and 120 M1V7 mutants against soft rot caused by *P. myriotylum* resulted in short listing three mutants without infection. Four mutants which escaped three rounds of *Ralstonia solanacearum* infection were clonally multiplied for further yield evaluation.

Comparison of the transcriptomes

To determine the effect of the infection by *R. solanacearum* on gene expression in mango ginger (*Curcuma amada*) and ginger (*Zingiber officinale*), both the transcriptomes were compared. A total of 20,938 *C. amada* and 20,061 *Z. officinale* genes were expressed. Differential expression analysis was performed using either reads per kilo base per million (RPKM) or count data. Based on three fold change and false discovery rate (FDR) P value <0.005, 1201 genes were identified as differentially expressed, out of which 587 genes were up-regulated and 613 genes were down-regulated. The up-regulated genes were classified into functional categories related to defense response, pathways and molecular function with respect to bacterial infection. Among the 54 differentially expressed transcription factors, 34 were up-regulated in *C. amada* which included WRKY, MYB, leucine zipper protein, zinc finger and GATA domain transcription factors. Genes involved in mevalonate pathway (MEP) for biosynthesis of isoprene/terpenes were found to be up-regulated substantially in *C. amada* compared to *Z. officinale*.

Source-sink relationship

The source-sink relationship in ginger was studied using three varieties *viz*., IISR Varada, IISR Rejatha and IISR Mahima. All the three varieties showed similar tillering and dry matter accumulation pattern with maximum number of tillers at 105 days after planting which coincided with rapid dry matter accumulation in rhizomes. Photosynthetic rate was maximum during 105-120 days after planting. Rhizome oil and oleoresin were low during the initial rhizome development and increased with rhizome dry matter accumulation. Rapid rhizome dry matter accumulation (75-120 DAP) had positive correlation with rhizome starch accumulation (rhizome bulking), photosynthetic rate and rhizome quality parameters.

Weed management studies

Field experiment conducted to compare various weed management practices on growth and yield of ginger (IISR Varada) revealed maximum yield (8t ha^{-1}) with the application of coir pith compost (4t ha^{-1}) + leaf mulches (7.5t ha^{-1}) at 45 and 90 day after planting which was on par with application of *Glycosmis pentaphylla* leaves (30t ha^{-1}) and *Lantana camara* leaves 30 t ha^{-1}. Among plastic mulches, spreading of ash coloured plastic mulch recorded less dry weight of weeds, maximum height of the plants, number of leaves and maximum yield (4.87t ha^{-1}) followed by white coloured plastic mulch.

Transplanting of ginger

A transplanting technique in ginger by using single bud sprouts raised in pro-trays was standardized (Fig. 13). The results of replicated trial with different treatments revealed no significant difference for fresh yield among single sprout transplanted and direct planting of 20-25g seed rhizomes. The advantages of this technology are production of healthy planting materials and reduction in seed rhizome quantity and eventually reduced cost on seeds.

Fig. 13. Transplanting ready ginger bud sprouts in pro-trays

Bacterial Wilt

Collection and characterization of *Ralstonia solanacearum*

Eleven new isolates of *R. solanacearum* from ginger, small cardamom and tomato were added to the repository; all the isolates were of biovar 3. The isolates were tested for their pathogenicity and wide variation was observed in the isolates; the days taken for infection varied from 6- 23 days.

Isolation and evaluation of phages

Four phages were isolated from ginger rhizosphere soil collected from Wayanad. The phages isolated from Wayanad (Kerala) were studied for disease suppression and the disease incidence was reduced to 13%-20% when compared to control.

Isolation and evaluation of apoplastic bacteria

A total of 150 bacteria were isolated from the apoplastic fluid of pseudostems and leaves of ginger collected from different areas and accessions. These were evaluated *in vitro* and *in planta* against *R. solanacearum* for biocontrol potential and six isolates *viz.*, IISR GAB 24, IISR GAB 42, IISR GAB 43, IISR GAB 48 IISR GAB 107 and IISR GAB 146 were found to be promising showing no infection after challenge inoculation.

Studies on endophytic bacteria

Pseudomonas putida BP-25 R::gfp showed excellent colonization on ginger and it could be detected in all the plant parts by dilution plating and by Bio-PCR. Highest number of colonies could be detected in the roots 14 days post inoculation. *Bacillus megaterium* colonized only the rhizoplane and roots of ginger. However, ginger plants pre-colonized with both these bacteria failed to give protection against *R. solanacearum*.

Rhizome rot

Encapsulation and field testing of PGPR

Trials on encapsulation and field testing of a plant growth promoting rhizobacteria (IISR GRB 35- *Bacillus amyloliquefaciens*) for growth promo-

tion and disease control indicated that application of GRB 35 cell suspension, 1 capsule 5 kg^{-1} seed and 2 capsules 5 kg^{-1} seed registered comparable yields (7.9, 7.6 and 7.8 kg $3m^{-2}$ bed, respectively). However, these yields were significantly greater than metalaxyl-mancozeb (4.0 kg $3m^{-2}$) and absolute control (3.3 kg/ $3m^{-2}$) (Fig. 14). The study revealed the efficiency of delivering PGPR through capsules (Fig. 15) for growth promotion and disease control. A patent for this delivery process has been filed and commercialization is in progress.

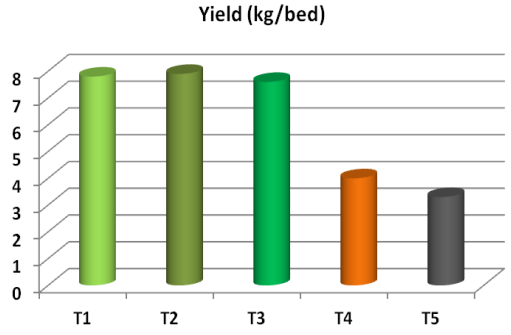


Fig. 14. Effect of PGPR delivery systems on ginger yield (T1) cell suspension (T2) 1 capsule/ 5 kg seed (T3) 2 capsules/ 5 kg seed (T4) metalaxyl-mancozeb and (T5) absolute control

Fig. 15. IISR GRB35 (*B. amyloliquefaciens* in gelatin capsules)

Shoot Borer

Evaluation of EPNs

The infectivity of four promising EPNs such as *Heterorhabditids* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius gingeri* (IISR-EPN 07) and *Oscheius* sp. (IISR-EPN 08) was tested against shoot borer larva (*Conogethes punctiferalis*) infesting ginger and turmeric under pot and field conditions. Liquid formulation of the EPN @ 50000 IJs pot^{-1} and 2 lakh IJs bed^{-1} were applied at 21 days interval from August to November. Among the test EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) treated plants showed minimum shoot damage in ginger (5.4 and 6.1%, respectively) and turmeric (21 and 28.6%, respectively) in comparison to control (34.1 and 40%, respectively) in the pot experiment. Whereas in the field, minimum shoot damage was recorded in ginger (22.9%) and turmeric (26.0%) when treated with *Steinernema* sp. (IISR-EPN 02) in comparison to control (47.5 and 50.4%, respectively), which was on par with malathion 0.1% treatment (17.4 and 25.3%, respectively).

Compatibility of EPNs with pesticides

The effect of malathion 0.1%, chloropyriphos 0.07% and mancozeb 0.3% on the activity of four EPNs *viz*., *Heterorhabditids* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *O. gingeri* (IISR-EPN 07) and *Oscheius* sp. (IISR-EPN 08) was studied. All the tested EPNs were compatible with malathion and chloropyriphos; however, mancozeb adversely affected the activity of *Heterorhabditids* sp. (IISR-EPN 01) and *Oscheius* sp. (IISR-EPN 08) (34% and 57% mortality, respectively).

TURMERIC

Genetic resources

One thousand four hundred and four *Curcuma* accessions have been maintained in the field gene bank. Germplasm conservatory was enriched with six turmeric accessions, which includes a unique *C. amada* accession from Andhra Pradesh with purple pigmentation in leaf midrib. Eighty seven accessions of turmeric varying in curcumin content were characterized as per the DUS guidelines.

Breeding

A multilocational trial with three promising turmeric accessions (Acc. 48, Acc. 79 and Acc. 849) along with IISR Prathiba and local check was laid out in Kerala (Peruvannamuzhi), Andhra Pradesh (Vijayawada), Tamil Nadu (Erode) and Karnataka (Appangala). In Vijayawada, Peruvannamuzhi and Erode, highest fresh yield was recorded in Acc. 48 and IISR Prathibha, whereas in Appangala, IISR Prathiba and Acc. 849 recorded highest fresh yield.

Fig. 16. A promising turmeric accession (Acc. 48)

Exploitation of other *Curcuma* species

Though there was not a significant variation for strach yield in four *Curcuma* species *viz., C. amada, C. aromatica, C. xanthorrhiza* and *C. caesia,* the starch granules of the four species differed in size, shape (Fig. 17) and solubility.

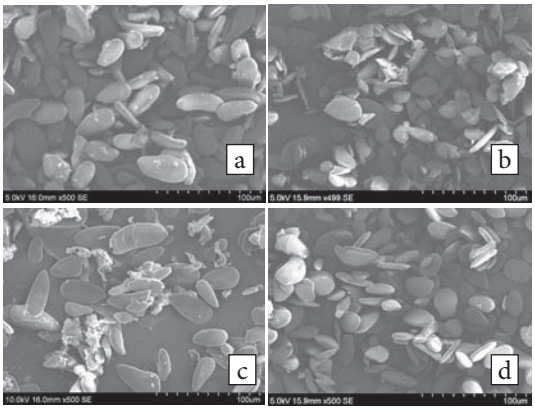


Fig. 17. Scanning electron micrograph of starch granules of four *Curcuma* species. a) *C. amada*, b) *C. xanthorrhiza*, c) *C. aromatica* and d) *C. caesia*

Molecular biology

A short and easy protocol for RNA isolation from turmeric was optimized.

curs genes isolation

A pooled normalized cDNA library from turmeric tissues was constructed and about 100 clones having insert size of 1-3 kb were sequenced and clones carrying isoforms of curcumin synthase (*curs* I, II and III) were identified. qPCR analysis confirmed expression of curcumin synthase isoforms from rhizome and leaf tissues.

Molecular markers

Sixty five new primers were designed and 17 polymorphic SSR markers were identified for screening turmeric accessions. Cross species amplification was found successful in other species of *Curcuma*, ginger and cardamom using all these markers (Fig. 18). Polymorphic markers CLM 33 could distinguish varieties Suguna and Sudarshana from the rest of the released varieties.

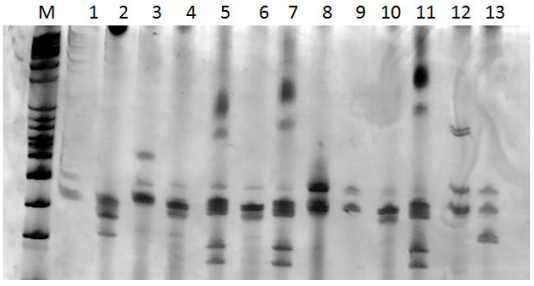


Fig. 18. Image showing PCR profiling of SSR primer CLM 36; M-100 bp DNA ladder, 1 - *C. pseudomontana*, 2 – *C. raktakanta*, 3 – *C. leucorhiza*, 4 – *C. manga*, 5 – *C. malabarica*, 6 – *C. amada*, 7 - *C. xanthorhiza*, 8 – *C. longa*, 9 – *C. caesia*, 10 – *C. aromatica*, 11 – *C. zedoaria*, 12 – *Zingiber officinale*, 13 – *Elettaria cardamomum*.

Source-sink relationship

IISR Alleppey Supreme and IISR Prathibha were studied for source-sink relationship. Though the dry matter accumulation pattern remained same in both the varieties, IISR Prathibha accumulated more dry matter and at a faster rate compared to IISR Alleppey Supreme. Rapid dry matter accumulation and rhizome bulking occurred be-

tween 90-135 days after planting. Maximum photosynthetic rate was noticed during 120-135 days after planting. Rhizome bulking, photosynthetic rate and IAA content had positive correlation with dry matter accumulation in rhizomes.

TREE SPICES

Genetic resources

A seedless nutmeg from Kottayam (Kerala) besides 14 monoecious nutmeg from Karnataka were collected and added to the genebank during the year.

Breeding

A nutmeg variety 'IISR- Keralashree' has been recommended for release by AICRPS during the year through farmer's participatory breeding. This variety has bold nuts with whole, thick reddish mace. The mace and nut are rich in sabinene and myrcene.

Molecular biology

In cinnamon, *rbcL* locus showed higher interspecific divergence while *psbA-trnH* exhibited lower interspecific divergence. SNPs specific to *C. aromaticum* (*C. cassia*) were detected in *rbcL* locus in two out of the five market samples studied thereby confirming the presence of *C. cassia* adulteration in commercial samples of true cinnamon. Out of the three loci (*matK, psbA-trnH and rbcL*), *rbcL* locus proved to be efficient in tracing out adulterants in traded cinnamon. *C. malabatrum* adulteration was not detected in any of the traded samples analyzed. These species specific SNPs could be exploited in designing species specific primers, enabling kit development for easy detection of adulterants. Barcodes of all the species generated have been deposited in NCBI database.

Quality profiling

Among 14 accessions of mace evaluated for essential oil profile, majority contained sabinene, pinenes, limonene, α terpineol and myristicin as chief constituents; IC 548921 (21.5% myristicin and 10.7% elemicin); IC 548918 (13.2% myristicin and 14.2% safrole); IC 645944 (18.2% safrole and 11.0% elemicin) were identified as unique accessions. The antioxidant activity of mace oil showed positive correlation with myristicin level.

Production of food extruders from selected spices

Extrusion studies on rice flour blended with spices was studied in a twin screw extruder. Extrudates from rice flour blended with dry ginger had the lowest water absorption index, compared to other extrudates with an average value of 4.21. Rice flour blended with dry ginger gave better extrudates based on their overall acceptability scores at a die temperature of 140°C and a screw speed of 350 rpm.

EXTENSION AND TRAINING

Seven meetings of the monthly technology advisory committee under Agricultural Technology Management Agency (ATMA), Calicut district were held at the Institute in which monthly technology advisories were prepared and passed on to extension agencies. The meeting was attended by block level Assistant Directors of Agriculture and ATMA field functionaries.

Two 'on demand on campus' training courses on production management and post harvest technology of spices sponsored by the Department of Agriculture and Food Processing, Uttarakhand and Department of Agriculture, Assam were organized. Ten officers from Uttarakhand and 15 from Assam participated.

The Institute participated in 10 off campus exhibitions/Farmers melas which includes, Krishi Vasant 2014 at Nagpur, exhibition in connection with the International Conference on Tuber Crops for Sustainable Livelihood at Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram and technology showcasing event under the NAIP project on mobilizing mass media support

for sharing agro information.

Mobilising mass media

Forty five news clippings and 13 success stories appeared in various English/Malayalam/Hindi newspapers/agriculture magazines/portals.

Eight radio talks through AIR, Kozhikode and four through Janavani FM, Kannur, Kerala.

Organised one technology showcasing exhibition with 30 stalls depicting technologies/ products by Government and Non governmental agencies. Farmers, students and general public visited the stalls.

Farmer's feed back

Feedbacks from farmers' plot revealed high yield of two released turmeric varieties Sivenesan, Gundlupet, Karnataka, 40t acre^{-1} for IISR Alleppey Supreme; Dr. Kailas R Pogare, Nanded, Maharashtra, 27t acre^{-1} for IISR Prathibha) (Figs. 19 & 20).

A front line demonstration of IISR Prathiba variety of turmeric was conducted in four farmers filed in Guntur district under the National Horticultural Mission. An average yield of 40 t ha^{-1} was recorded in the demonstration plots. To synergize the adoption process of improved varieties of turmeric and make farmers updated on scientific cultivation a two day training programme was organized at Vijayawada during 21-22 January, 2014 under the National Horticulture Mission in which 75 farmers from various districts of Andhra Pradesh attended. The training was organized in collaboration with AICRPS centres at Guntur and Kamrapally.

Fig. 19. Mr. Sivenesan (extreme right) in his Alleppey Supreme plot

Fig. 20. IISR Prathibha plot of Dr. Kailas R Pogare, Nanded, Maharashtra

Front line demonstration of IISR Prathibha conducted at Guntur, Andhra Pradesh proved to be a big success as about 80 farmers participated in the programe.

ITM-BPD UNIT

Two Entrepreneurship development programmes, one business meet and one workshop on Intellectual Property Rights (Fig. 21) were conducted and prospective entrepreneurs were identified for commercialization of micronutrient technology. Six patent applications were filed. One brochure on Business Planning and Development (BPD) Unit, IISR and one folder on technologies for commercialization were published. Two Entrepreneurship Development Programmes (EDP) organized at IISR attracted around 150 participants each and six people were enrolled in the BPD Unit. One license has been issued for commercialization of ginger variety IISR Varada. In collaboration with the Kerala Agricultural University, trials have been initiated for testing of the "Seed coating composition technology" on vegetable seeds.

Fig. 21. Programme on IP and its management for growth and prosperity conducted in collaboration with NRDC, New Delhi

KRISHI VIGYAN KENDRA

About 151 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted and 5139 trainees were benefitted. Eleven front line demonstrations and six 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. Two gardeners' training programmes of six months duration sponsored by State Horticulture Mission were organized empowering 50 rural youth. Three farmers/farmer groups also received National awards including the IARI Innovative Farmer's Award during the period in recognition of their achievements in the field of agriculture. Besides, 676 plant-animal clinic consultancy services, 41200 vaccinations of poultry birds and animals and two animal health campaigns were conducted. Participatory seed production on high yielding varieties of ginger and turmeric was also taken up in 4 farmers plots. About 32 Short Message Service (SMS) and 13 voice message on latest updates on agriculture and allied fields were sent to 743 farmers and 100 Extension functionaries. The Kendra also conducted 15 seminars, participated in 10 Kisan Mela cum exhibitions, broadcasted four radio talks and three farmers study tours. Technology week was celebrated during 21-24 January 2014 during which a one day awareness programe on PPV&FRA, honouring three innovative farmers, quiz and elocution for school students etc. were conducted. During this year Rs. 14.08 lakhs has been realized through sale of various technological inputs to farmers.

HUMAN RESOURCES DEVELOPMENT

Trainings conducted

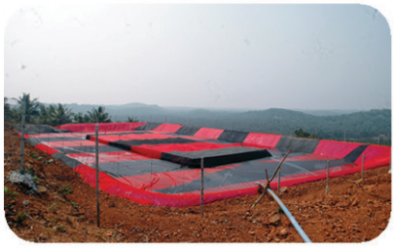
- Next generation sequencing: Data analysis and annotation, 17-20 March 2014
- Developing institutional repositories using DSpace, 12-13 March 2014
- Information literacy in the digital era, 12 August 2013

M.Sc. / Ph.Ds

One student was awarded with Ph.D. from Mangalore University

NEW FACILITIES

- Infrastructure facilities for healthy planting material production of spices were created with about 640 m^2 each of fully controlled and naturally ventilated poly houses.
- The BPD office and incubation facilities were established.
- A High Performance Computing (HPC) facility was established under the PhytoFuRa project for the analysis of next generation sequence (NGS) data. PhytoFuRa-HPC is a cluster of three nodes with GPU accelerators and high speed (InfiniBand) connections between nodes. Each node has 8 processing cores, 48GB of RAM, and Intel Xeon processors.
- Besides creation of 25 lakh litre water harvesting structure at Chelavoor farm, five acres of land was cleared and terraced for undertaking field experiments



भाकृअनुप
ICAR

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किसानों का हमसफर
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