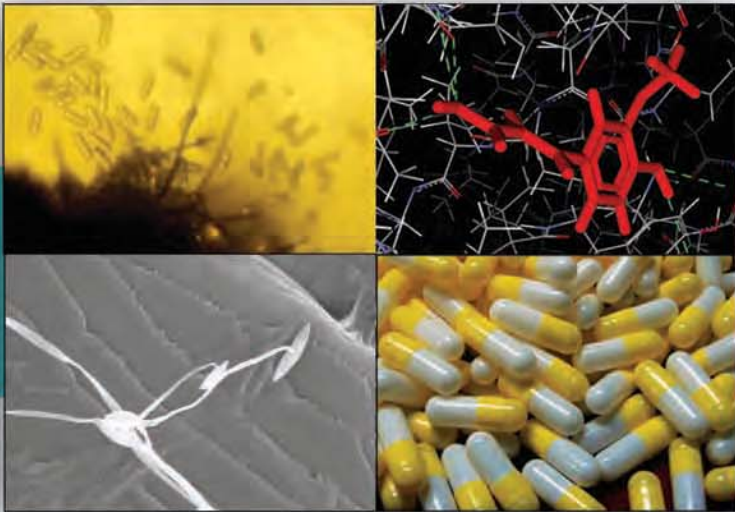
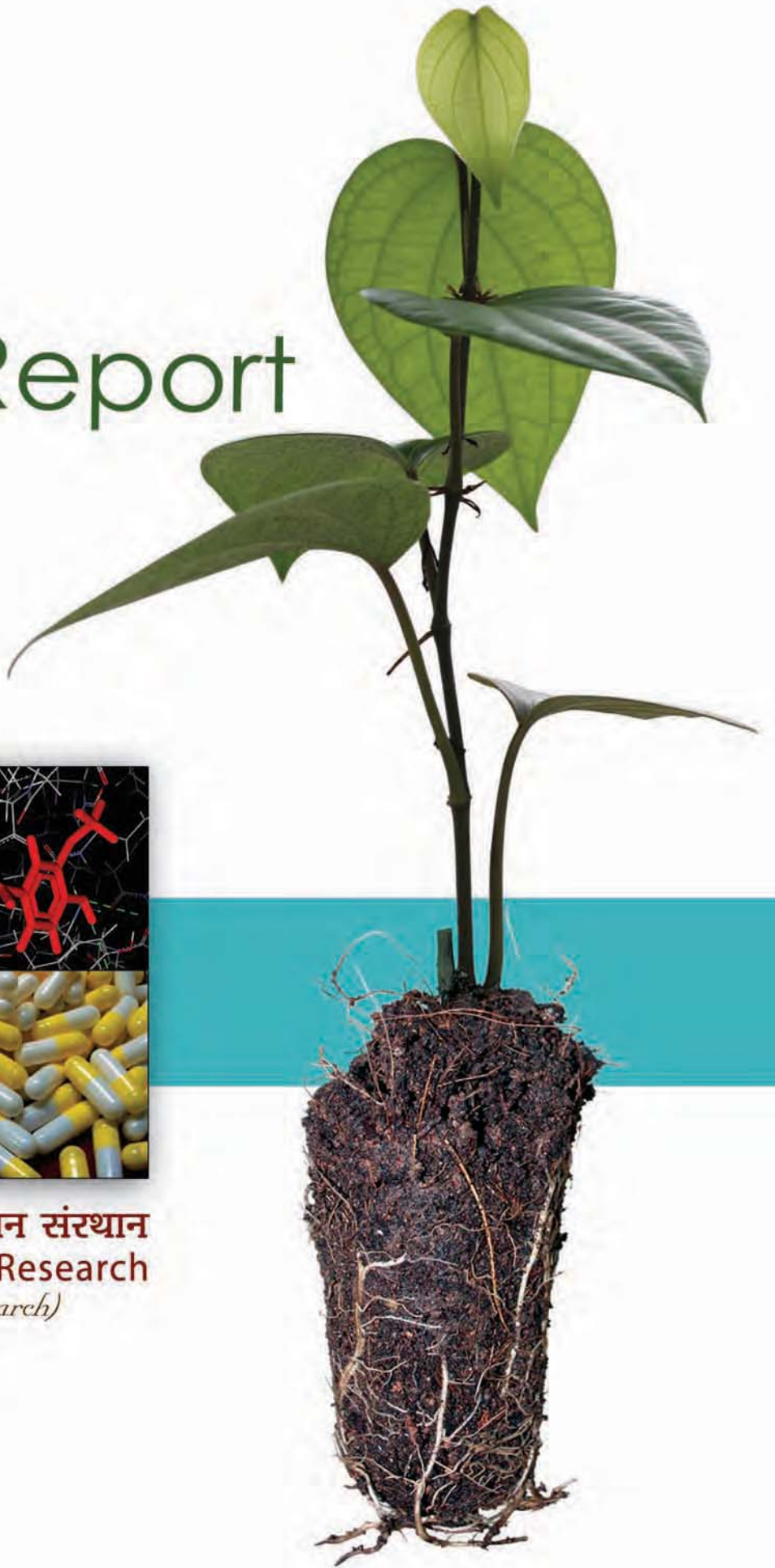




Annual Report

2013/14



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



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

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2013/14

ANNUAL REPORT

2013/14



भाकृ अनुप
ICAR

भारतीय मसाला फसल
अनूसंधान संस्थान
कोषिकोड



Indian Institute of
Spices Research
Kozhikode



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PREFACE

The research achievements of the Institute during 2013/14 is presented here as Annual Report. During this year, collections were made to enrich the cultivar diversity in black pepper from Sirsi, Yellapur, Honnavar, Sagar regions of Karnataka. In nutmeg, a seedless nutmeg from Kottayam (Kerala) and 14 monoecious nutmegs from Karnataka are notable collections during the year. A nutmeg variety 'IISR- Keralashree' developed through farmer's participatory breeding has been recommended for release by AICRPS. 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 (P). 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 transplanting (single sprout) were standardized and passed on to extension agencies

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*) from 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 facilities for healthy planting material production of spices were 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. Malhotra, 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.

Kozhikode
15.06.2014

(M. Anandaraj)



EXECUTIVE SUMMARY

BLACK PEPPER

Genetic resources and breeding

Five wild relatives and 21 accessions were collected from Sirsi, Yellapur and Honnavar Taluks of Uttar Kannada and Sagar region of Shimoga district. The present status of black pepper germplasm accessions conserved at the gene bank is 3181 (wild pepper - 1503, cultivars - 1669, exotic species - 9). A germplasm block consisting of 142 accessions was established at Central Horticulture Experimental Station (CHES), Chettalli as an alternate center. Improved varieties and examples varieties (DUS) were planted for conservation and top shoot production under protected condition. IC numbers were obtained for 224 accessions.

Hybridizations were undertaken using Subhakara as female parent and bold berried accessions *viz.*, Vadakkan and Waynadan Bold 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 40-51% and 78- 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 open reading frame (ORF) finder revealed that 39 out of 51 could be translated into a single 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.

Targeted expression analysis of resistance genes

The R genes (NBS4 and NBS5) expression pattern by qPCR was different between resistant (IISR Sakthi) and susceptible (Subhakara) lines suggesting that R genes have a distinct pattern of expression and play a critical role in *Phytophthora 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 post inoculation (hpi) 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 dehydration responsive element binding (DREB) was studied using qPCR. The genes showed significantly higher expression in tolerant variety under stress, the maximum being observed in case of dehydrin. The expression analysis of these three genes suggested that drought tolerance is associated with a rapid modulation of genes from different gene families.

Proteogenomics

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

Tissue culture

Meristem culture technique using liquid culture medium for the production of plantlets from 2.0 mm shoot tips was standardized. A regeneration protocol via direct shoot bud formation was standardized using greenhouse grown leaf explants of *P. colubrinum*. Maximum number of shoots was produced from leaf discs cultured on half strength MS medium II supplemented with 2 mg L⁻¹ BA and 0.01 mg L⁻¹ NAA. Plant regeneration and rooting of the plants took four months from culture initiation.

Soil carbon sequestration

Soil samples were collected (0-25 cm) from high density cropping systems having coconut, banana, nutmeg, cinnamon and black pepper as component crops 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 basin (11.6 g kg⁻¹ and 35.2 g kg⁻¹) followed by nutmeg and coconut. The total organic N content also was found to be higher under black pepper basin 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. About 61% of the samples registered high (25-35 kg ha⁻¹) to extremely high (100 kg ha⁻¹) available P levels.

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 nursery growth 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 cuttings.

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 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 5th 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 48h.

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. 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. The market samples were, however, free of *P. galeatum* and *P. attenuatum*



(wild species) adulteration. *rbcL* and *rpoC1* could differentiate *P. atteinatum* from *P. nigrum* and *P. galeatum* while *psbA-trnH* differentiated *P. galeatum* from *P. nigrum* and *P. atteinatum*. Adulteration even at very low levels (0.5%) could be detected using barcoding locus.

Phytophthora foot rot and slow decline

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* making the total collection to 442.

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, while 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 revealed SNP sites; common genes with reference genome of *P. capsici* (JGI), and genes unique in *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

Two new strobilurin fungicides viz., Ergon 44.3% (w/w) [Kresoxim methyl 500 g L⁻¹] and RIL-070/FI (72WP) were evaluated *in planta* against *P. capsici*. Ergon was evaluated at 5000-8000 ppm concentrations and 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 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 under green house conditions for growth promotion and disease suppression. Growth promotion was promising in consortia containing Act 2+5, Act 2+9 and Act 5+9.

Evaluation of Trichoderma isolates

Geographically different isolates of *Trichoderma* 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 PhytoFuRa8 and PhytoFuRa15 (65.5% and 63.38% disease control, respectively) when compared to control (85.6% disease incidence).

Evaluation of chemicals against Radopholus similis

Nematicidal activity of five chemicals viz., fipronil (10 and 15g pot⁻¹), thiamethoxam (0.5 and 1g pot⁻¹), carbosulfan (G) 5 and 10g pot⁻¹ and carbosulfan (0.1% and 0.2%) were evaluated against *R. similis* under pot conditions among which fipronil (15g 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 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. *In vitro* screening of these compounds 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 to *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 48h 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 96h. *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.

Complete genome sequencing of *Piper yellow mottle virus* (PYMoV)

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 7549-7607 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 adnavirus 1*, *Cacao swollen shoot virus* and *Citrus yellow mosaic virus*.

Genetic diversity

The conserved reverse transcriptase / ribonuclease 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.

Influence of temperature on symptom expression

Symptomless PCR positive and negative plants of black pepper cuttings were exposed to 35°C, 60% RH for 8h 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 2D electrophoresis coupled with mass spectrometry analysis yielded major host proteins which will have influence on symptom expression.



Anthracnose

Epidemiology of anthracnose

Studies on activation of microsclerotia of *Colletotrichum gloeosporioides* in runner shoots of black pepper showed that the microsclerotia were activated within seven days when subjected to high humid conditions *in vitro*.

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. The leaf bit with exudate when inoculated on the black pepper variety, Panniyur-1, resulted in the formation of typical anthracnose symptoms. Pathogenicity of the cultures was proved on variety 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 under field conditions.

CARDAMOM

Genetic resources and breeding

A total of 618 accessions have been maintained in the National Active Germplasm Site (NAGS) at Cardamom Research Centre (CRC), Appangala. Sixty accessions were characterized for yield and yield contributing characters. Natural incidence of leaf blight (*Colletotrichum gloeosporioides*) 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.

Screening of germplasm for identification of sources of resistance to cardamom thrips (*Sciothrips cardamomi*) was continued for the third consecutive year in association with Indian Institute of Horticultural Research, Bengaluru. Two hundred and seventy eight accessions were screened during the year. Twenty six accessions recorded below 20% total capsule damage. IC 349455 recorded the lowest capsule damage of 8.3%. All these accessions belonged to *Malabar* type. IC 349582, a *Vazhukka* type showed highest damage of 98.5%.

A hybrid (NH₃₅) has been shortlisted for high yield and *katte* resistance. Twenty one inter-varietal F₁ hybrids are shifted to main field for yield studies. Also, 23 selfed progenies have been shifted to main field for thrips tolerance studies.

Standardizing the parameters for target 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 P₂O₅ and 14.8 for K₂O (Green Gold) and 17.3% for N, 7.3% for P₂O₅ and 8.3% for K₂O (Appangala-1). The nutrient contribution from fertilizer was worked out to be 26.6% for N, 4.35% for P₂O₅ and 15.2% for K₂O (Green Gold) and 11.4% for N, 2.7% for P₂O₅ and 7.1% for K₂O (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

Nursery validation of management strategies

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 under nursery conditions.

Field validation of management strategies

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 application of *T. harzianum* thrice at 30 days interval was promising in reducing leaf blight incidence under field conditions.

Rhizome–root rot

Identification of primary causal organism

Inoculation studies with *Pythium vexans*, *Rhizoctonia solani* and *Fusarium oxysporum* individually and in combination on 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 4h to colonize the roots, whereas *R. solani* and *F. oxysporum* required 12 and 96h, respectively. Under high humid conditions, sporangia of *P. vexans* were produced in abundance and aggregated near the root tip region. *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.

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*, carboxin + thiram 0.2% and captan + hexaconazole 0.2% were effective under *in vitro* conditions. Carboxin + thiram 0.2% and tebuconazole 0.05% were effective against *R. solani* whereas, tebuconazole 0.05% was superior against *F. oxysporum* under laboratory conditions.

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

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 for the management of thrips in association with Indian Institute of Horticultural Research, Bengaluru. The trial indicated that among the treatments, fipronil (1.0 mL L⁻¹), quinalphos (2 mL L⁻¹), Spinosad (0.3 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), Spinosad (0.3 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 used in the study 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

The 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 β -tubulin and partial translation elongation factor 1 α 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. 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 of spice crop pests

Surveys were conducted in 75 locations in nine districts in 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 *Protospulvinaria* 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. nigrinus* were recorded on mussel scale infesting black pepper.

GINGER

Genetic resources and breeding

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

Evaluation of extra bold and low fibre accession led to the identification of three accessions (Acc. 723, Acc. 247, Acc. 278) with high yield and bold rhizomes.

Four thousand one hundred and twenty 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 to take up 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, 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 RPKM or count data. Based on three fold change and FDR P value <0.005, 1201 genes were identified as differentially expressed, out of which 587 genes were up-regulated and 613 genes are 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 on 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 variety IISR Varada revealed maximum yield (8 t ha^{-1}) with the application of coir pith compost (4 t ha^{-1}) + leaf mulches (7.5 t ha^{-1}) at 45 and 90 day after planting (DAP), which was on par with application of *Glycosmis pentaphylla* leaves (30 t 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 (4.87 t ha^{-1}) followed by white coloured plastic mulch.

Transplanting

A transplanting technique by using single bud sprouts raised in pro-trays was standardized. The results of replicated trial with different treatments revealed no significant difference for fresh yield among single sprout transplanted and direct planting of 20-25 g seed rhizomes. The advantages of this technology are production of hi-health planting materials and reduction in seed rhizome cost.

Bacterial Wilt

Collection and characterization *Ralstonia solanacearum*

Eleven new isolates of *R. solanacearum* from ginger, small cardamom and tomato were added to the repository; all of the isolates were 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 isolated phages isolated were studied for disease suppression

and the disease incidence was reduced to 13-20% when compared to control.

Isolation and evaluation of apoplactic bacteria

A total of 150 bacteria were isolated from the apoplactic fluid of pseudostems and leaves 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 in the plant after challenge inoculation.

Studies on endophytic bacteria

Pseudomonas putida BP-25 R::gfp showed excellent colonization 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, 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 GRB35-*Bacillus amyloliquefaciens*) for growth promotion 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 $7.8 \text{ kg } 3\text{m}^{-2}$ bed, respectively). However, these yields were significantly greater than metalaxyl-mancozeb ($4.0 \text{ kg } 3\text{m}^{-2}$) and absolute control ($3.3 \text{ kg } 3\text{m}^{-2}$). The study revealed the efficiency of delivering PGPR through capsules for growth promotion and disease control. A patent for this delivery process has been filed.

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 during 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 is on par with malathion 0.1% treatment (17.4 and 25.3%, respectively).

Compatibility of EPNs with pesticides

The effect of malathion 0.1%, chloropyrifos 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 chloropyrifos; however, mancozeb adversely affected the activity of *Heterorhabditids* sp. (IISR-EPN 01), *O. gingeri* and *Oscheius* sp. (IISR-EPN 08) (34% and 57% mortality, respectively).

TURMERIC

Genetic resources and breeding

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

A multilocational trial with three promising 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 Prathiba, whereas in Appangala, IISR Prathiba and Acc. 849 recorded highest fresh yield.

curs genes isolation

A pooled normalized cDNA library from tissues were constructed and about 100 clones having insert size 1-3 Kb were sequenced and identified clones carrying isoforms of curcumin synthase (*curs* I, II and III). 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 accessions. Cross species amplification was found successful in other species of *Curcuma*, ginger and cardamom using all these markers. Polymorphic markers CLM 33 could distinguish varieties Suguna and Sudarshana from the rest of the released varieties of turmeric.

Source sink relationship

IISR Alleppey Supreme and IISR Prathiba were studied for source sink relationship. Though the dry matter accumulation pattern remained same in both the varieties, IISR Prathiba accumulated more dry matter at a faster rate compared to IISR Alleppey Supreme. Rapid dry matter accumulation and rhizome bulking occurred between 90 and 135 DAP. Maximum photosynthetic rate also was noticed at between 120 and 135 days after planting. Rhizome bulking, photosynthetic rate and IAA content had positive correlation with rapid dry matter accumulation in rhizomes.

TREE SPICES

Genetic resources and breeding

A seedless nutmeg from Kottayam (Kerala) and 14 monoecious nutmegs from Karnataka were collected and added to the genebank during the year. 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. malabattrum* adulteration was not detected in any of the traded samples analyzed. Barcodes of all the species have been deposited in NCBI database.

Quality profile of some selected nutmeg accessions

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 extrudates 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 or ajowain would give 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 the 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 were organized sponsored by the Department of Agriculture and Food Processing, Uttarakhand and Department of Agriculture, Assam. Ten officers from Uttarakhand and 15 from Assam states participated.

The institute participated in 10 off-campus exhibitions/Farmers mela including Krishi Vasant 2104 at Nagpur, the All India agriculture cum trade fare organized by the Ministry of Agriculture and Cooperation; Exhibition in connection with the International conference on tuber crops for sustainable livelihood at CTCRI, Thiruvananthapuram and technology showcasing event under the NAIP project on mobilizing mass media support for sharing agro information

Mobilising mass media support for sharing agro-information

About 45 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 were given. One technology showcasing exhibition with 30 stalls depicting technologies/ products by Government and Non governmental agencies was organized.

Farmer's feed back and FLD

Feedbacks from farmers' plot revealed very high yield for released turmeric varieties. A front line demonstration (FLD) of IISR Prathiba variety of turmeric was conducted in four farmers fields 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 to update farmers 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.



INSTITUTE TECHNOLOGY MANAGEMENT-BUSINESS P L A N N I N G A N D DEVELOPMENT UNIT

Two entrepreneurship development programmes (EDP), one business meet and one workshop on Intellectual Property Rights were conducted and prospective entrepreneurs were identified for commercialization of micronutrient technology. Six patent applications were filed. One brochure on BPD Unit, IISR and one folder on technologies for commercialization were published. The two EDP programmes 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.

KRISHIVIGYAN KENDRA

About 151 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted and 5139 trainees were

benefitted. Eleven FLDs 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. 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 in recognition of their achievements. 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 four 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, broadcast four radio talks and three farmers study tours. Technology week was celebrated during 21-24 January 2014 during which a one day awareness programme on PPV&FRA, honouring three innovative farmers, quiz and elocution for school students etc. were conducted. During this year Rs. 14.08 lakhs was realized through sale of various technological inputs to farmers.



INTRODUCTION

History

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

Location

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

Mandate

- ◆ To extend services and technologies to conserve genetic resources of spices as well as soil, water and air of spices agroecosystems.
- ◆ To develop high yielding and high quality spice varieties and sustainable production and protection systems using traditional and non-traditional techniques and novel biotechnological approaches.
- ◆ To develop post harvest technologies of spices with emphasis on product

development and product diversification for domestic and export purposes.

- ◆ To act as a centre for training and technology upgradation of spices and to coordinate national research projects.
- ◆ To monitor the adoption of new and existing technologies to make sure that research is targeted to the needs of the farming community.
- ◆ To serve as a national centre for storage, retrieval and dissemination of technological information on spices.

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

Organization

The Director is the administrative head of the institute. The Institute Management Committee, Research Advisory Committee and Institute Research Committee assist the Director in matters relating to management and research activities of the institute. Research on various aspects of the mandate crops is conducted in three divisions, namely, Division of Crop Improvement and Biotechnology, Division of Crop Production and Post Harvest Technology and Division of Crop Protection and a Social Sciences Section. The other facilities available at the Institute include Agricultural Technology Information Centre, Agricultural Knowledge Management Unit, Bioinformatics Centre and Krishi Vigyan Kendra. The Institute also functions as the headquarters for the All India Coordinated Research Project on Spices (AICRPS), and Indian Society for Spices (ISS). An



outreach project on *Phytophthora*, *Fusarium* and *Ralstonia* diseases of horticultural and field crops (PhytoFuRa) was sanctioned in the XI plan (2007-12) with IISR, Kozhikode as the lead centre and 17 coordinating centres at different ICAR institutes/SAUs across India. The institute has also linkages with several universities, research institutes, and developmental agencies for collaborative research and developmental activities in spices.

Budget

The total budget of the institute was 1926.64 lakhs during the year, which included 600.00 lakhs (including OPR on *PhytoFuRa*) under Plan and 1278.64 lakhs under Non Plan.

Resource generation

Institute earned a total of 9.86 lakhs through sale of planting materials, biocontrol agents, training, publications and consultancy services.

Staff

The institute has a sanctioned strength of 44 scientific, 24 administrative, 31 technical and 33 supporting staff, of which 33, 19, 28 and 33 of scientific, administrative, technical and supporting staff, respectively are in position. The KVK has a sanctioned strength of 2 administrative, 12 technical and 2 supporting staff.

Staff position of the Institute

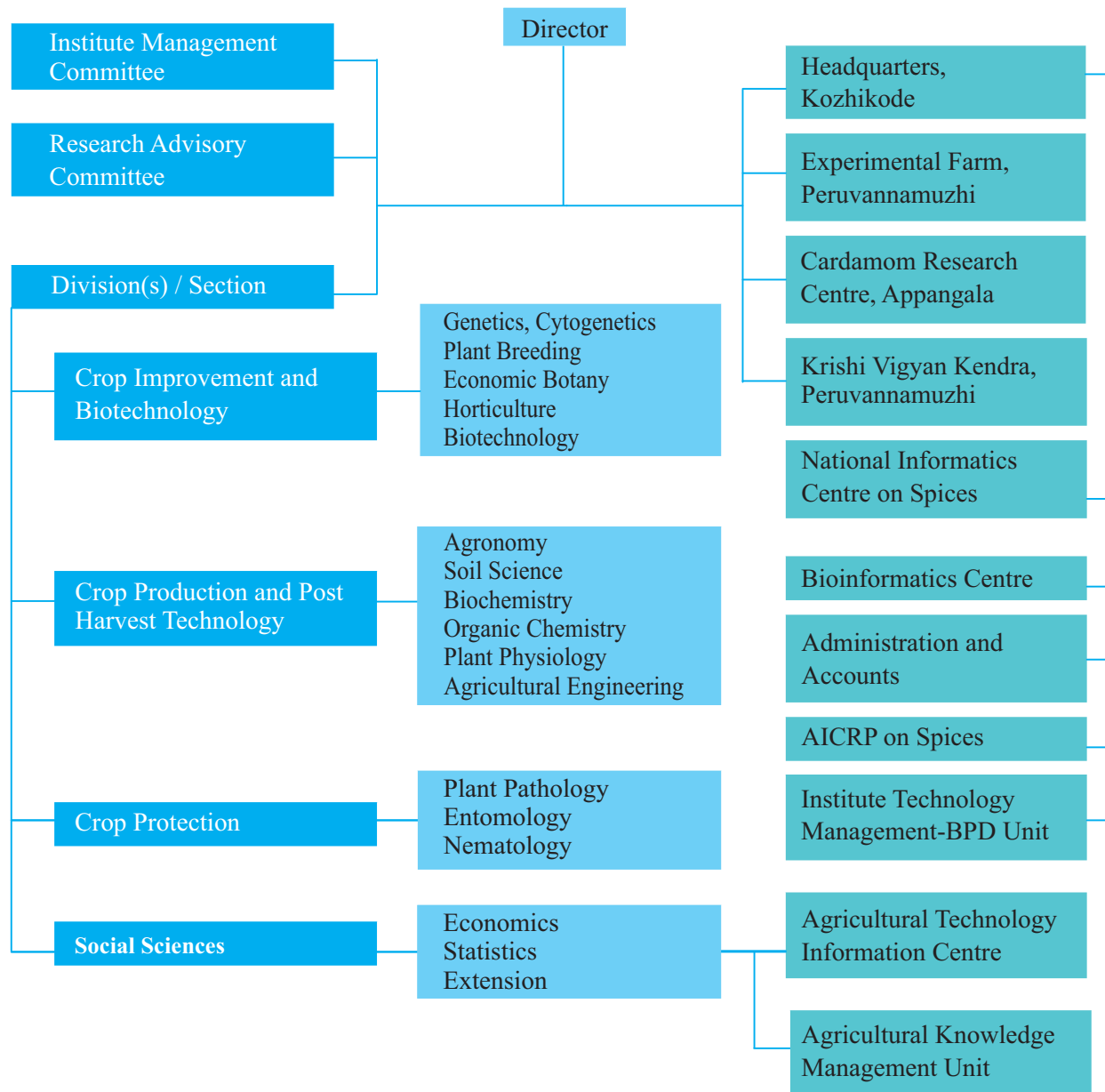
Category	Sanctioned	Position			Total	Vacant
		Kozhikode	Peruvannamuzhi	Appangala		
Scientist	44	28	1	4	33	11
Technical	31	14	10	4	28	3
Administration	24	17	-	2	19	5
Supporting	33	10	7	16	33	-
Total	132	69	18	26	113	19

Staff position of KVK

Category	Sanctioned	Position			Total	Vacant
		Kozhikode	Peruvannamuzhi	Appangala		
Technical	12	-	12	-	12	-
Administration	2	-	1	-	1	1
Supporting	2	-	2	-	2	-
Total	16	-	15	-	15	1



Organizational chart





Past Achievements

Black pepper

Germplasm collections obtained over the years through explorations are being maintained at IISR as well as in other alternate sites *viz.*, Appangala and Chettali of Karnataka for developing improved varieties for yield, quality, abiotic and biotic stresses. The genetic stock has led to the release of nine improved varieties such as Sreekara, Subhakara, Panchami, Pournami, PLD-2, IISR Thevam, IISR Girimunda, IISR Malabar Excel and IISR Shakthi. FLD programme was undertaken using the released varieties in the farmers' field. Two accessions, INGR 8099- *P. thomsonii* (IC 398863) - for its unique character for sex change and INGR 8100- *P. nigrum* (IC 563950) – a novel spike variant with proliferating spikes, were registered with NBPGR, New Delhi for their unique characters. Endangered species *viz.*, *P. barberi* and *P. hapnium* were located and collected from Sabari hills. Microsatellites developed for *Piper* species were successfully used to detect polymorphism in black pepper cultivars. Assembly and functional annotation of sequences derived from the transcriptome of *P. colubrinum* and *P. nigrum* helped in the identification of many genes involved in defense and secondary metabolism. Seedlings of *P. colubrinum* on screening with *P. capsici* showed segregation of the resistance character, 21 plants being resistant to *Phytophthora*, 2 plants susceptible and the rest showing moderate resistance. Putative transgenic black pepper plants with osmotin gene conferring resistance to drought and *Phytophthora capsici* has been developed. *In vitro* and *in vivo* propagation methods were standardized. Plantlets developed through micropropagation were established in farmers' field in Kerala and Karnataka.

The spacing, nutrient and water requirements were standardized for different soil types of pepper growing regions. Irrigating pepper vines once in a fortnight from March to May months at the rate of 50 litres/vine enhanced yield substantially. High production technologies and mixed cropping systems were developed for increasing productivity. Among different forms of potash, water-soluble and available

K had significant positive correlation with berry yield, oleoresin and piperine. Organic production technology for black pepper has been standardized. Crops such as ginger, tapioca, coleus, amorphophallus and hybrid napier were found suitable for intercropping in black pepper gardens that are more than 15 years old. Intercropping medicinal plants (*Vetiveria zizanoids* and *Alpinia calcarata*) in juvenile black pepper garden was found to be profitable with a B:C ratio of 2.3. Cost effective method for production of disease-free rooted cuttings was developed. A machine was fabricated in collaboration with CIAE, Coimbatore centre which is capable of mixing, pulverizing, sieving, and filling of potting ingredients in poly bags at desired quantity. Mathematical models for optimum climatic factors for high production of black pepper have been developed. Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations in black pepper. Major pests, pathogens, viruses and their insect vectors and nematodes affecting pepper were characterized and documented. Morphological and molecular characterization of black pepper isolates of *Phytophthora* further revealed that isolates shared the characters of both *P. capsici* and *P. tropicalis*.

A RNA virus, *Cucumber mosaic virus* (CMV) and a DNA virus, *Piper yellow mottle virus* (PYMoV) are found to be associated with stunted disease of black pepper. A method for simultaneous isolation of RNA and DNA from infected black pepper plants and multiplex PCR for simultaneous detection of CMV and PYMoV in a single reaction was standardized. SYBR green based real-time PCR was developed for detection of PYMoV and CMV in black pepper. Phytoplasma with phyllody symptoms was most closely related to members of aster yellows group (16Sr I) of Phytoplasma. Integrated strategies involving cultural methods, biocontrol agents, plant products and resistant varieties were developed for the management of pests and diseases including nematodes that resulted in substantial increase in yields and pesticide free produce. Large scale multiplication of biocontrol agents such as

Trichoderma and *Pseudomonas* for distribution to farmers for management of disease was also undertaken. The open pollinated progeny of IISR Shakthi 04-P24-1 continued to be resistant to root infection by *P. capsici* even after 4 years after planting in the field. These organisms were deposited in the national repository of microorganisms at IMTECH, Chandigarh for future reference. Species-specific primers were developed for detection of *R. similis* in soil and plant samples. The presence of β -1, 4 endoglucanase, a major secretory cellulose enzyme in nematodes, was located in *R. similis* through EST analysis. Black pepper accessions, HP-39 and Acc. 1090 were found to be resistant to nematodes besides being rich in caryophyllene. Endophytic bacteria effective against *Phytophthora capsici* and *R. similis* in black pepper have been isolated. Culture filtrates of BRB 13 at 40 μ L mL⁻¹ caused 100% mortality of *R. similis* within 24h. Basal application of *T. harzianum* and aerial spray with 1% Bordeaux mixture was found effective in controlling anthracnose disease. An integrated pest management schedule for management of root mealy bug has been developed. Metalaxyl-MZ sensitivity of 81 phytophthora isolates was tested and the EC₅₀ and EC₉₀ values ranged from 0.0002 to 14.4 ppm and 1.1-68.5 ppm, respectively. Among the new chemicals tested *in vitro* against *P. capsici*, Acrobat 50 showed 100% inhibition at 50 ppm concentration. Profiling and activity prediction of biochemical compounds using *in silico* tools were completed for *Pseudomonas putida* BP 25 and *Bacillus megaterium* BP 17. PCR based techniques were developed for identification of traded black pepper and to detect adulterants in commercial black pepper powder. The existence of fungicide sensitive or resistant isolates among the field populations of *C. gloeosporioides* infecting black pepper was noticed in Pollibetta and the isolate from this locality was tolerant to recommended doses of Bordeaux mixture and carbendazim. Post harvest technologies for drying, processing, storage and production of value-added product like white pepper production were standardized.

Genomic DNA was isolated from 126 black pepper *Phytophthora* isolates and SSR profiling was done. Genetic diversity of *Phytophthora* isolates from black pepper was studied by ITS sequencing with the universal primers ITS 6 and ITS 4. A native isolate of

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

Impact studies on adoption of IISR varieties of black pepper in farmers' fields indicated that the mean yield for high yielding varieties was 1160 kg ha⁻¹ with the adoption of scientific packages as compared to 620 kg ha⁻¹ for traditional varieties. The estimated cost benefit ratio was 2.48. The level of adoption studies of recommended technologies indicated that the adoption level for aerial spraying of Bordeaux mixture for the control of fungal diseases was 57.14% and for application of biocontrol agents was 64.2%. The adoption level for application of soil fungicides, fertilisers and pesticides were very low at 21.14%, 7.7% and 7.6 % respectively. *Karshika Sankethika Darshanam* and Media Meet were organized to mobilize mass media support for sharing Agro-Information. Video films on Augmenting black pepper production – a success story (Malayalam, English, Hindi) and success story of a 'Prathiba' grower – post production stage were produced.

Cardamom

Germplasm collections obtained over the years through explorations are being maintained at CRC, Appangala. IC numbers have been obtained for all the available germplasm. Meanwhile, germplasm bearing unique characters have been registered with NBPGR, New Delhi. The improved varieties such as Appangala-1, IISR Vijetha and IISR Avinash have been developed. Coupled with production technologies, these varieties resulted in increasing productivity of cardamom. About 10 high yielding F1 hybrids were promoted to future coordinated varietal trials. Molecular profiles were developed for 100 accessions of small cardamom germplasm using 25 ISSR markers for studying the



genetic diversity and dendrogram of similarity was prepared. Molecular profiling of Indian cardamom revealed the existence of two genetically distinct clusters such as “Kerala cluster” and “Karnataka cluster’ among the germplasm collections. Characterization of export grade cardamoms from India, Sri Lanka and Guatemala based on physical, biochemical parameters and molecular techniques revealed the superiority of Indian produce. GC-MS study confirmed superiority of Indian cardamom over Guatemalan and Sri Lankan cardamom. High production technology has been standardized. Drip irrigation and sprinkler irrigation once in 12 days significantly improved yield attributing characters. Soil and water conservation measures have been standardized in cardamom based cropping system. Cardamom accessions APG 257, APG 414 and APG 434 were found to be promising for drought tolerance.

A procedure for total RNA isolation and detection of CdMV through reverse transcription–polymerase chain reaction (RT-PCR) using primers designed for the conserved region of coat protein was standardized. A protocol for SYBR green based real-time RT-PCR for detection of *Cardamom mosaic virus* (CdMV) and *Banana bract mosaic virus* (BBrMV) in cardamom was developed. Surveys conducted in Karnataka and Kerala, revealed the prevalence of *Banana bract mosaic virus* (BBrMV) infection. A reliable RT-PCR based method was also developed for detection of the virus in plants. The survival of *C. gloeosporioides* infecting cardamom in infected plant part (leaves) was studied under laboratory, greenhouse and field conditions. A new bacterial wilt disease on small cardamom was noticed in Wayanad, Kerala. Phenotypic and genetic characterization revealed that the causative organism is *R. solanacearum* biovar 3 phylotype 1. Multiplex-PCR based phylotyping, 16s rDNA & recN gene sequence based comparison and MLST based comparative genetic analysis further revealed that the strain is 100% similar to the ginger strain of *R. solanacearum*.

Ginger

Germplasm repository at IISR is the largest collections with several exotic collections and high quality accessions. Six hundred and sixty eight accessions are being maintained in field germplasm

conservatory. Three varieties namely, IISR Varada, IISR Rejatha and IISR Mahima were released for high yield and quality. Cross specific amplification of rice microsatellites was successfully done in ginger. Acc. 195, a tetraploid having $2n=44$, showed mean pollen fertility of 67.73% by glycerol-carmin staining and 60.31% by *in vitro* germination and is suitable for future studies on induction of seed set. Two accessions irradiated with gamma rays showed resistant reaction after three repeated inoculations with *R. solanacearum*. Ginger oil components have been characterized by GC-MS. A relationship between leaf P/Zn ratio and soil P/Zn ratio to rhizome yield has been established. Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations. The economic optimum in terms of profitable response for money invested was found to be Rs. 3.75 bed^{-1} for N, Rs. 1.30 bed^{-1} for P and Rs. 0.60 bed^{-1} for K.

Post harvest technologies for processing and technologies for preparation of value added products such as salted ginger were standardized. Comparison of essential oil constituents of fresh and dry rhizomes indicated that fresh rhizomes contained higher level of monoterpenes namely, *Z*-citral and *E*-citral whereas the dry rhizomes were predominated by the sesquiterpene hydrocarbons *viz.*, zingiberene, farnesene and sesquiphellandrene. Ginger strain of *R. solanacearum* was found to infect turmeric, cardamom, *C. aromatica*, *C. zedoaria*, *Kaempferia galanga*, *Zingiber zerumbet* and tomato. Indian mango ginger, *Curcuma amada* was found to be free from bacterial wilt even under inoculated conditions. The species of *Pythium* causing rhizome rot of ginger in Kerala, Karnataka, Uttar Pradesh and Sikkim was identified as *P. myriotylum*. Nine actinomycete isolates from ginger soil were found to be antagonistic to *R. solanacearum*. Technique for ginger seed rhizomes treatment (for elimination of bacterial wilt pathogen) and integrated disease management strategy for soft rot and bacterial wilt diseases and shoot borer was developed. *Bacillus amyloliquefaciens* (GRB 35) was effective for disease control and plant growth promotion. PGPR formulation to enhance nutrient mobilization and growth, yield and biocontrol was developed and commercialized. The life cycle of shoot borer

(*Conogethes punctiferalis*) was studied on six resistant and six susceptible accessions. The infectivity of EPNs strains IISR-EPN 01 to 08 was tested against shoot borer larvae under *in vitro* conditions. One species of EPN belonged to *Oscheius gingeri* and was identified as new species on the basis of morphological and molecular characterization. The improved varieties and technologies developed on cropping system, nutrient and water requirement, pest and disease management and post harvest processing techniques were disseminated to farmers and other agencies through publication, training programmes and demonstrations. Large scale multiplication and distribution of elite planting material were also undertaken.

Turmeric

The germplasm collected over the years have been conserved in the field gene bank and were characterized for yield, quality, and resistance to pests, diseases and drought. Seven high curcumin and high yielding varieties, Suvarna, Sudarsana, Suguna, IISR Prabha, IISR Prathiba, IISR Alleppey Supreme and IISR Kedaram were released for commercial cultivation. Open pollinated seedling progenies generated over the years are being evaluated for their yield and quality characters. Molecular genetic fingerprints of sixteen *Curcuma* species using RAPD and ISSR technique revealed high degree of polymorphism among the accessions. A total of 140 microsatellites containing genomic DNA fragments were isolated adopting the selective hybridization method with di and trinucleotide biotinylated probes. Two synonymous *Curcuma* species viz., *C. zedoria* and *C. malabarica* showed identical SSR profiles for 40 microsatellite loci. Efficient protocol for plant regeneration through organogenesis and somatic embryogenesis was standardized. Variations in rhizome morphology were observed among calli-regenerated somaclones indicating somaclonal variation. Accessions with high curcumin and root knot nematode resistance were identified. About 40 seedling progenies with higher curcumin (> 3%) and dry recovery (> 20%) were identified. The natural enemies of shoot borer (*Conogethes punctiferalis*) were documented. Three different curcuminoids (curcumin, de methoxy curcumin and bis de methoxy

curcumin) could be separated from oleoresin by employing chromatographic techniques. Turmeric oil components have been characterized by GC-MS. A PCR based method was developed to detect adulteration of turmeric powder with wild *Curcuma* species.

Targeted yield equations for predicting nutrient requirements for fixed yield targets in soils with varying fertility levels were standardized with minimum deviations. The economic optimum in terms of profitable response for money invested was found to be Rs. 0.65 bed⁻¹ for N, Rs. 0.40 bed⁻¹ for P and Rs. 0.85 bed⁻¹ for K. Increase in curcumin content was recorded when sprayed with micro nutrients like zinc and boron. Processing with or without boiling or different drying methods did not lead to variation in oil, oleoresin and curcumin contents. The optimum spacing, nutrient and water requirement were standardized for different soils and organic farming system was developed for turmeric. Basic data on distribution, bioecology, population dynamics of shoot borer (*Conogethes punctiferalis*) and its natural enemies and crop loss due to shoot borer was generated. Lamda cyhalothrin 0.0125% was more promising in reducing the percentage of shoots infested by the shoot borer. The improved varieties and technologies were disseminated to farmers and other agencies through publications and demonstrations. The adoption of released varieties like IISR Prathiba in Andhra Pradesh, Karnataka and Tamil Nadu were studied. Novel soil pH based micronutrient mixtures for enhancing growth, yield and quality of turmeric, ginger, black pepper and cardamom were developed.

Tree spices

The germplasm holdings of three important tree spices, nutmeg, clove, cinnamon including cassia, garcinia and allspice are being conserved. IC Numbers for cinnamon, clove, nutmeg and allspice accessions were obtained from NBPGR, New Delhi. Cassia C1 (IC 370415) has been registered as INGR 05029 with NBPGR, New Delhi for its high oleoresin content (10.5%) besides a dwarf clove accession. The cassia elite line A1 (IC 370400) has been registered with NBPGR for high cinnamaldehyde content in bark oil



(81.5%) and leaf oil (80.5%). Two high quality cinnamon varieties, Navashree and Nithyashree and a nutmeg variety, Viswashree were released. Nutmeg accession, A11/25 was found to be promising for high yield. Nutmeg accession A9-71 (IC-537220), as a source of high sabinene (45.0% sabinene in nutmeg oil and 41.9% sabinene in mace oil) was registered with NBPGR. Tissue culture protocols have been developed for nutmeg. Protocols for DNA isolation from nutmeg have been standardized. Performance of nutmeg on *M. malabarica* continued to be better than other rootstocks for productivity. Green chip budding with orthotropic buds was standardized in nutmeg on *Myristica fragrans* rootstock with 90-100% success. GC-MS study revealed the presence of two chemotypes in *Cinnamomum verum*. Drying and processing methods for cinnamon, nutmeg and mace have been developed. Antioxidant properties and food color value are being studied in tree spices. GC-MS analysis of the chemical constituents of essential oils in leaves of *Cinnamomum sulphuratum*, *C. glaucescens*, *C. glanduliferum*, *C. macrocarpum* and *C. perrottetti* revealed that the major chemical constituents in these oils were α -phellandrene, β -phellandrene, camphor, *t-caryophyllene* and *germacrene-D* respectively. Vegetative propagation techniques were standardized for nutmeg, cassia and cinnamon. Major pests and diseases on tree spices were documented. The improved varieties and technologies developed on propagation and post harvest processing were disseminated to farming community. Four species of *Garcinia* viz., *G. kydia* (Kuji Thekera), *G. lancifolia* (Rupohi Thekera), *G. pedunculata* (Bor Thekera) and *G. xanthochymus* (Tepor Tenga) were located in Meghalaya, Assam and Nagaland. Hot water extraction and Solvent extraction (Methanol/chloroform -1:1) of *G. gummigutta* and *G. tinctoria* yielded 50% butter with yellow colour and pleasant aroma.

Vanilla

Vanilla germplasm are being maintained in the repository, which includes a flower colour variant collected from Andaman and Nicobar islands.

Comparative anatomical analysis of different vanilla species was carried out. Interspecific hybridization was made between *Vanilla planifolia* and *V. aphylla*. Reciprocal crosses were conducted between *V. planifolia* and *V. tahitensis* (species reported as resistant to root rot disease) and high percent of fruit set was observed in both the crosses. Fifty interspecific hybrids each of *V. planifolia* \times *V. tahitensis*, *V. tahitensis* \times *V. planifolia* and selfed progenies of *V. tahitensis* were established *ex vitro*. Chromosome number analysis of two interspecific hybrids between *V. planifolia* and *V. tahitensis* showed $2n=30$ in one (PT-5) and $2n=32$ in other (PT-17).

Protocols for micro propagation through direct shoot multiplication as well as callus regeneration were standardized. Root rot and wilting were found to be the major problems in most of the plantations. Root rot incidence ranged from 5 to 100%. Mosaic and necrosis were also observed in all the plantations and the incidence ranged from 2 to 80%. *Cucumber mosaic virus* (CMV) of vanilla was characterized on the basis of biological and coat protein (CP) nucleotide sequence properties, which showed that CMV infecting vanilla belongs to subgroup IB. A virus causing mild chlorotic mottle and streaks on leaves of vanilla was identified as a strain of *Cymbidium mosaic virus* (CymMV) based on coat protein gene sequence comparison and phylogenetic studies. Another virus associated with necrosis and mosaic on vanilla was identified as a strain of *Bean common mosaic virus* (BCMV) based on coat protein gene sequence comparison and phylogenetic studies.

Paprika

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

RESEARCH ACHIEVEMENTS

BLACK PEPPER

Genetic resources

Diverse cultivars were collected from farmer's plots of three states viz., Kerala, Karnataka and Tamil Nadu. A total of 99 collections were made including 96 cultivars and three wild *Piper* species.

Unique accessions collected

An accession with very high dry recovery (46%)



was collected from a farmer's plot at Agali (Fig. 1).

Fig. 1. Acc. 7452 - An accession with high dry recovery collected from farmer's plot at Agali, Palaghat, Kerala.



Fig. 2. Acc. 7547 and Acc. 7548, long spike accessions collected from Wayanad, Kerala

Two local types having very long spikes but with poor setting were located from one of the estates in Wayanad (Fig. 2) and conserved. These accessions were characterized and evaluated.

IC numbers were obtained for 224 accessions established in the conservatory last year. With this all the accessions collected and established up to 2012 and conserved in the germplasm conservatory were allotted IC numbers from NBPGR, New Delhi. The present status of germplasm conserved at the conservatory is 3181 accessions consisting of 1669 cultivars, 1503 related taxa and nine exotic species. These accessions are maintained at the National Active Germplasm Site (NAGS) at the experimental farm, Peruvannamuzhi. About 175 accessions were planted in the field. Improved varieties and examples varieties of DUS were planted for conservation and production of orthotropic shoots under protected condition and 142 accessions were planted at the field genebank at the alternate center, CHES, Chettalli. Also, 160 accessions were collected and maintained under controlled climatic conditions at IISR, Kozhikode.

Breeding

An yield trial comprising of selected entries viz., Hp 1411, Hp 780, Hp 728, Hp 39, Coll. 1114, Coll. 820, Coll.1090, Opkm, Thommankodi, Hp1117, Thevam (control) and Sreekara (control) were planted at Peruvannamuzhi. Ten plants of the progeny of P24 too were planted. Four of the entries flowered within six months of planting.

Screening of hybrids for pollu beetle resistance

Eighty plants of Karimunda x Acc. 816 were screened for pollu beetle damage on the leaves. The number of leaves damaged by pollu beetle and the area fed in each damaged leaf was recorded. Among the 80 plants screened, leaves of only three plants were damaged by pollu beetle.

Screening hybrids and mapping populations

Thirty eight open pollinated progenies of IISR Shakthi were screened for *Phytophthora* resistance. Among the 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 at 72h of inoculation. Thus, progenies more susceptible and more tolerant than the parent IISR Shakthi could be identified. Thirteen genotypes of the association mapping population were morphologically characterized based on DUS guidelines of PPV & FRA, New Delhi. The ISSR profiling of association mapping population is in progress. Profiling using 10 primers were completed; 16 SSR markers developed in black pepper were tested in five genotypes (Subhakara, Sreekara, P24-O-4, IISR Shakthi, *P. colubrinum*).

Screening against PYMoV

Out of 2342 germplasm accessions screened for resistance against PYMoV, four accessions showed resistance in the preliminary test.

Screening for drought tolerance

Sixty five accessions were screened for drought tolerance and significant differences was noticed for RWC and membrane leakage. Relative water content (%) in tolerant genotypes (Accs. 5606, 5616, 5624) after 14 days of stress was 74.3-74.5% and in susceptible genotypes (Accs 5621, 5655, 5657) it ranged from 58.4-60.4% and cell membrane leakage in tolerant genotypes ranged from 8.2-8.7 and in susceptible genotypes it ranged from 15.1-18.2.

Tissue culture

Meristem culture technique was applied to produce virus free plants in variety Sreekara.



Fig. 3. Black pepper plantlets through meristem culture

Technique using liquid culture medium for the production of plants from 0.2 mm shoot tips was standardized (Fig. 3). The viral indexing showed that plantlets were positive to virus.

Regeneration protocols for *P. colubrinum*

A regeneration protocol via direct shoot bud formation was standardized using green house grown leaf explants of *P. colubrinum* (Fig. 4). The basal medium composition as well as the 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) concentration for direct organogenesis from leaf explants was evaluated and three of the tested media gave plantlet regeneration by means of direct shoot bud formation. The regenerated shoot buds were elongated on full strength MS medium with same hormone concentration and rooted in hormone free half strength MS medium II (containing macro and micro nutrients at half strength). Maximum number of shoots was produced from leaf discs cultured on half strength MS medium II supplemented with 2 mg L⁻¹ BA and 0.01 mg L⁻¹ NAA. Plant regeneration and rooting of the plants took around four months from culture initiation.

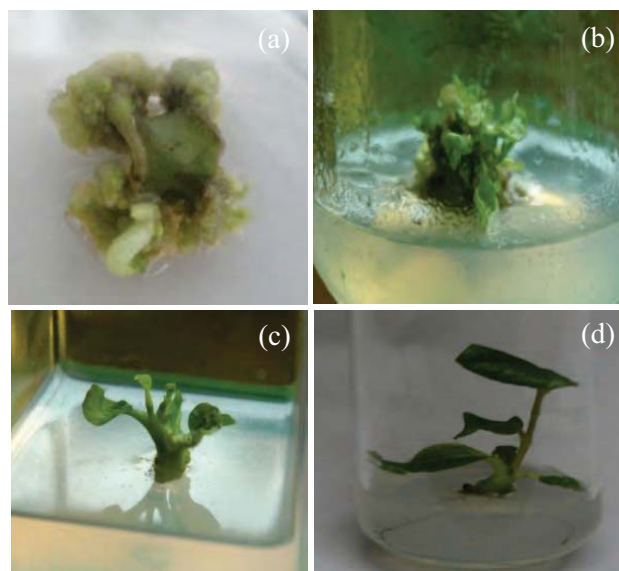


Fig. 4. *In vitro* shoot bud formation and plantlet regeneration from leaf explants of *Piper colubrinum* (a) shoot bud induction at the cut end of leaf (b) multiple shoot elongation (c) single shoot transferred to rooting medium (d) rooted plantlet

Host–pathogen interaction

Isolation of resistance gene candidates

Isolation of resistance gene candidates was attempted from IISR Shakthi, Sreekara, Subhakara, an open pollinated progeny of IISR Shakthi (O4-P24) and two wild species viz., *P. colubrinum* (Acc. 392) and *P. ornatum* (Acc. 3362) using degenerate primers (oligonucleotide) designed from known R-genes. PCR amplification resulted in the production of 500 bp amplicon in all the plant materials. A total of 550 colonies were obtained upon transformation among which 50 colonies were sequenced. The level of identity of these sequences to RGAs isolated from other plant species ranged from 40-51% and 78-99% similarity to other *Piper* RGA sequences.

RNA was isolated from challenge inoculated IISR Shakthi (resistant) and Subhakara (susceptible) with *P. capsici* (05-06), at different time intervals (0.5, 1, 2, 4, 8, 12, 16, 24 h post inoculation), and the analysis of expression of R genes (NBS4 and NBS5) by qPCR was done. The expression pattern was different between resistant and susceptible varieties suggesting that R genes had a distinct pattern of expression and plays a critical role in *P. capsici* 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 susceptible. The higher level of transcript suggests that it is likely to be responsible for the large part of resistance to *P. capsici* (Figs. 5 & 6).

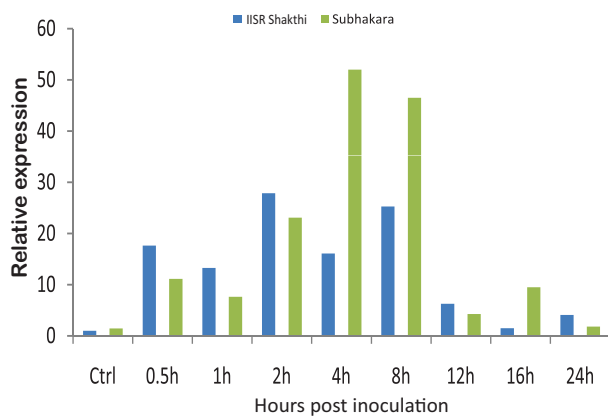


Fig. 5. Expression of R gene CBNS4

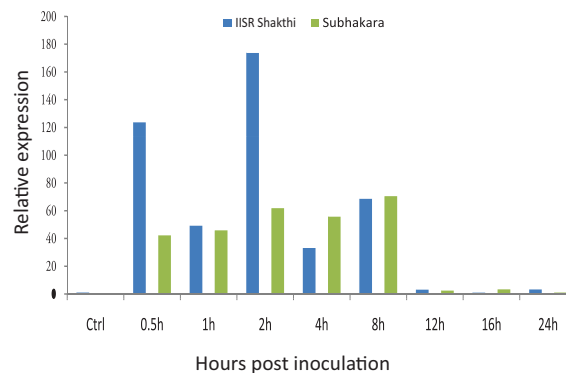


Fig. 6. Expression of R gene CBNS5

Transcription factors and defense genes in *Piper colubrinum*

The induction of defense related and other genes such as osmotin, β -1,3-glucanase, defensins, polygalacturonase inhibitor protein and phenylalanine ammonia lyase, were studied using real time PCR in *P. colubrinum* challenge inoculated with two strains (05-06 and 98-93) of *Phytophthora* and plants inoculated with 05-06 strain showed high level of expression for the genes.

Expression profiling of R-genes in *P. colubrinum*

The expression level (fold change) of the three putative R genes; LR 2277, LR 1990 and PCR 07 was investigated at different hours post inoculation (hpi) with two *P. capsici* strains (05-06 and 98-93). Highest level of expression was noticed in case of LR 1990 when challenged with *Phytophthora* isolate 05-06 while LR 2277 gene expressed maximum when challenged with isolate 98-93. The maximum expression of putative R gene, LR 2277 was in the initial period of pathogen interaction and there was a decrease in expression with time whereas, the expression of the other two genes LR 1990 and PCR 07 was maximum at 16 hpi (Fig. 7).

Leaf protein map for IISR Shakthi

A rapid method for total protein extraction was developed followed by highly reproducible 2D electrophoresis. Protein profiles were generated with both PH 3-11 and 4-7 IPG strips. Image analysis with Image Master Platinum 6.0 yielded good quality

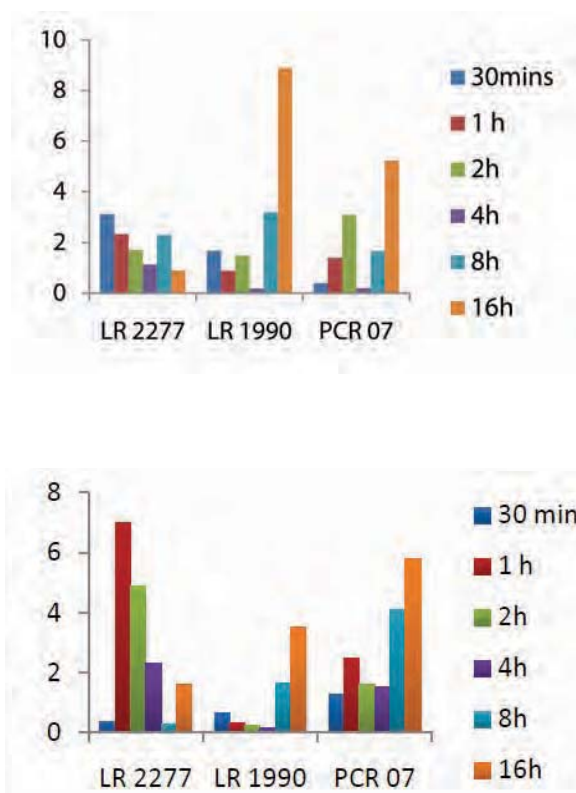


Fig 7. Expression pattern of the three putative R genes when challenge inoculated with *Phytophthora capsici* (a) 05-06 and (b) 98-93.

protein spots starting from saliency value at 10 onwards. With the fixed saliency of 1000, 15 spots were excised, subjected to in-gel digestion followed by LC-MS/MS Analysis. Mass spectral data were analyzed using MASCOT 2.4 on Proteome Discoverer 1.4. All 15 proteins were identified with tremble database with molecular weight and PI. Rec A like NTPase protein-novel transmembrane protein, chaperons (heat shock proteins), sugar kinase, actin, ATP synthase beta subunit, subunits of rubisco activase, subunits of ribo kinase, epo x hydrolase like protein, thiamine thialose synthase, subcellular oxygen evolving proteins and manganese stabilizing proteins. The spectrum of proteins represented all kind of protein groups viz., membrane proteins, sub cellular proteins, kinases, stress responsive proteins, heat shock proteins, plant defense related proteins and proteins involved in metabolism and photosynthesis.

2D proteomics coupled with mass spectrometry yielded many proteins with various biological significance viz., ATP synthase subunit (membrane protein), heat shock protein 60-2, heat shock protein 60 family, NBD sugar kinase hsp 70 family, putative actin family protein, ATP synthase beta subunit (fragment), rubisco activase (AAA superfamily), kinase (membrane protein), chloroplast rubisco activase (AAA Superfamily), fructokinase (ribokinase), epoxy hydrolase, chloroplastlipocalin, glyoxalase and proteosome subunit alpha type rubisco large chain (rbcl) (Fig. 8).

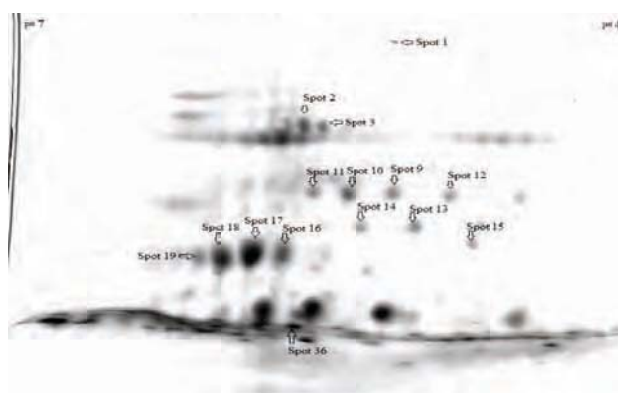


Fig. 8. LTQ-Orbitrap LC-MS: Linear Trap Quadrupole-Orbitrap-Liquid Chromatography-Mass Spectrometry

Drought stress

Coding sequences deduced from the nucleotides of the specific transcripts identified from the transcriptome data for dehydrin, osmotin and DREB protein were of 344,92 and 130 amino acids. Conserved domain searches through NCBI blastp suite and Interproscan confirmed the presence of motifs specific for dehydrin, antifungal thaumatin-like proteins in case of Osmotin and AP2 DNA-binding domain in case of DREB protein. These specific gene sequences had good match with similar sequences from other plants. The analysis using PROSITE, a consensus pattern, [KR]-[LIM]-K-[DE]-K-[LIM]-P-G corresponding to k sequence, which is a signature motif that is diagnostic of dehydrin proteins was found in the Dehydrin sequence.

With the aim of identification of water deficit stress-induced genes of functional relevance, the expression level of genes viz., dehydrin, osmotin and a

regulatory protein, DREB were studied using Real-time quantitative PCR. These genes studied showed significantly higher expression in the tolerant variety under stress, the maximum expression being observed in case of dehydrin. The transcript level expression of dehydrin was much higher in the drought induced tolerant variety Acc. 4216 (3571 fold) than in the drought susceptible variety Sreekara (108 fold) when compared to the respective control (well watered) plants. This suggested that drought tolerance is associated with a rapid modulation of genes from different gene families.

Assessment of cropping systems for the soil C buildup

Soil samples collected (0-25 cm) from high density cropping system having coconut, banana, nutmeg, cinnamon and black pepper component crops and their C buildup in terms of total organic carbon (TOC) and particulate organic carbon (POC) indicated that POC and TOC contents were higher under black pepper basin followed by nutmeg and coconut. POC constituted 18-33% of the TOC content. The total organic nitrogen (TON) content also was found to be higher under black pepper basin followed by coconut. Particulate organic nitrogen constituted 1.1-9.7% of the TON content under different component crops.

Management of virus affected black pepper gardens for yield sustainability

Trials were taken on the management of virus affected black pepper gardens for recouping its health and sustaining the yield at three estates in Madikeri district of Karnataka with five treatment combinations viz. T1- FYM + Fertilizers as per recommendation, T2- T1 + Micro nutrient spray (twice), T3- T1 + PGPR soil application, T4 – T3+ Micronutrient spray (twice) and T5 – Control. The black pepper vines (predominantly var. Panniyur-1) were graded for their virus infection status as mild, moderate and severe based on their visual symptoms and canopy coverage and treatments were imposed in two splits, once in June – July and second in August - September. Vines with moderate virus symptom category showed lower content of leaf nitrogen, magnesium, iron and zinc as compared to mild category at the initial stage. After six months of treatment imposition, the leaf concentration

of N, Fe and Zn reached levels equal to that of mild category vines. The spike intensity was also observed to be high in all the four treatments as compared to control. Visual observations also indicated masking of the viral symptoms on the vines under both the



Fig. 9. Virus management trial at Palonjee Estate, Chethalli, Madikeri; a. Moderate viral infected vine (June 2013) b. Production of new leaves and masking of symptoms (Feb 2014)

categories with more new leaves produced with less or no symptoms (Fig. 9).

Standardized soil-less nursery mixture for black pepper multiplication using plug-trays

Among the different nursery media combinations studied for black pepper multiplication, coir pith with *Trichoderma* and vermicompost recorded significantly higher nursery growth parameters than all other treatments (Fig. 10). It was confirmed that composted coir pith with vermicompost and *Trichoderma* is an ideal potting medium for black pepper nursery. Among the single node cuttings with three different maturities (collected from the serpentine method runners), maximum nursery growth 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 cuttings. It is indicated that the rooted cuttings with full leaf from middle and top portions recorded higher growth parameters.



Fig. 10. Healthy black pepper planting material production using pro-trays

Cryogenic grinding on biochemical properties

Black pepper var. Panniyur-1 was ground at 10°C and -50°C using mills of varying screw speed and tested for quality. About 20% reduction in oil was noted in the sample ground at 10°C compared to grinding at -50°C (Fig. 11). Oleoresin and piperine showed only marginal variation. Total phenol (Fig. 12) and antioxidant property did not show variation in relation to screw speed or temperature.

Cryoground (CG) and hammer mill ground (HMG) black pepper powder was stored at ambient temperature for about two months and samples were drawn at 10 days interval and tested for quality.

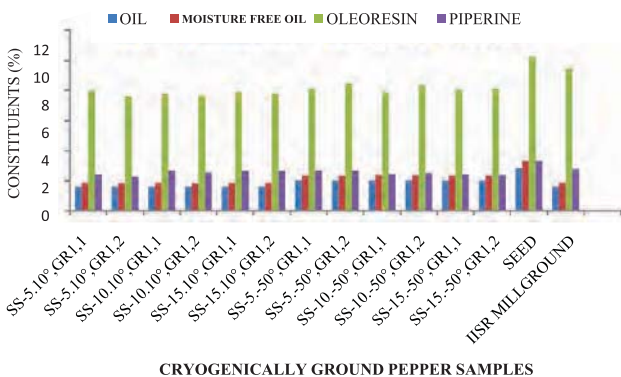


Fig. 11 . Quality profile of cryoground black pepper

Cryoground powder on storage showed reduction in oil from 30 days onwards while hammer mill ground sample did not show variation in oil content on storage for two months. Hammer mill ground sample showed 20% reduction in oil compared to freshly ground sample (Table 1). Oleoresin and piperine did not show

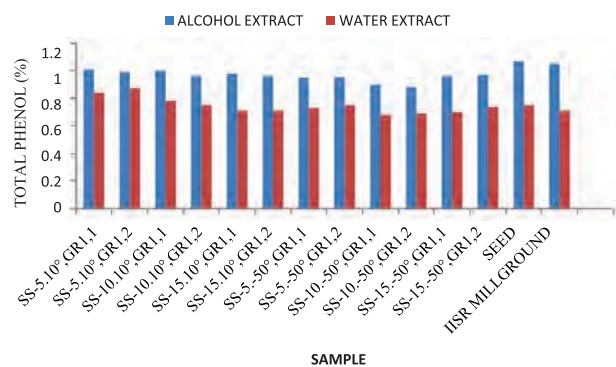


Fig . 12. Total phenol of cryoground black pepper

much variability between both the methods of grinding. Partial reduction in pinene, limonene of essential oil was observed during storage in cryoground sample (Fig. 13).

Production of white pepper

Experiments on production of white pepper from freshly harvested green pepper was conducted by

using four important bacteria viz., *Bacillus subtilis* (MTCC 5405, MTCC 5406 and MTCC 5407) and skin was obtained on the 6th day when cleaned and washed manually. Decortication was also completed

Table1. Quality profile of ground black pepper on storage at ambient temperature

Type of sample	Sample Name	Moisture (%)	Oil (%)	Moisture free oil (%)	Oleoresin (%)	Piperine (%)
Fresh	Seed	9.35	2.4	2.65	10.01	3.77
	HMG	8.07	2.0	2.18	9.04	3.46
	CG	9.55	2.4	2.65	9.10	3.52
After 10 days	Seed	8.58	2.4	2.63	9.31	3.67
	HMG	8.80	2.0	2.19	8.94	3.37
	CG	9.33	2.4	2.65	8.76	3.41
After 20 days	Seed	8.68	2.4	2.63	8.92	2.69
	HMG	9.22	2.0	2.20	8.84	2.56
	CG	9.47	2.0	2.65	8.65	2.63
After 30 days	Seed	9.42	2.4	2.65	8.91	2.67
	HMG	9.29	2.0	2.20	8.84	2.55
	CG	9.46	2.0	2.21	8.74	2.61
After 40 days	Seed	8.81	2.4	2.63	8.87	2.66
	HMG	9.16	2.0	2.20	8.83	2.55
	CG	9.43	2.0	2.21	8.74	2.61
After 50 days	Seed	9.05	2.4	2.64	8.79	2.63
	HMG	9.64	2.0	2.21	8.805	2.54
	CG	9.51	2.0	2.21	8.72	2.60
After 60 days	Seed	9.41	2.4	2.65	8.79	2.62
	HMG	9.59	2.0	2.21	8.76	2.50
	CG	9.40	2.0	2.21	8.70	2.55

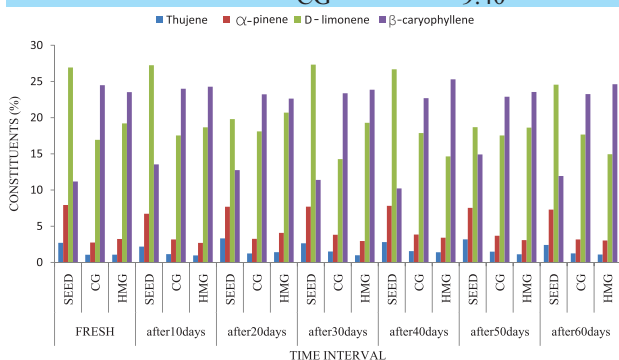


Fig 13. GC profile of cryoground black pepper Panniyur-1 on storage at ambient temperature

Bacillus licheniformis (MTCC 5408) at optimised conditions of 1.25 mL of 24h old culture 5 kg⁻¹ green pepper in ¼ strength of nutrient broth at 30-35°C. The results of the study indicated that pectinase enzyme production was maximum (120.5 Units mL⁻¹ of medium) on the 5th day when *Bacillus subtilis* (MTCC 5406) was used and complete decortication of outer

on the 6th day when the bacterial culture of *B. subtilis* (MTCC 5407) was used.

The enzyme assay of the above four bacterial cultures were performed in the pectinase enzyme production medium enriched with micronutrients incubated at three different temperatures of 30, 40 and 50°C for four days. The study indicated that *Bacillus subtilis* - MTCC 5407 showed the highest enzyme activity of 208.2 Units mL⁻¹ within 24 h at 40°C followed by *Bacillus subtilis* - MTCC 5406 and MTCC 5405 with enzyme activity of 197.4 and 102.4 Units mL⁻¹, respectively. Under similar conditions of temperature, *Bacillus licheniformis* (MTCC 5408) recorded the lowest enzyme activity (52.83 Units mL⁻¹) even after 48h.

Phytophthora foot rot and slow decline

Diversity and diagnostics

ITS sequencing of *P. capsici* isolates revealed two distinct groups among the isolates. Group I had close



resemblance to *P. capsici* isolates deposited in NCBI, whereas those of Group II had resemblance to both *P. tropicalis* and *P. capsici*. Multigene analysis was also done for characterization and differentiation of *Phytophthora* isolates. Nine genes viz., 28S ribosomal DNA, 60S ribosomal protein L10, beta-tubulin, elongation factor 1 α , enolase, heat shock protein 90, TigA gene fusion protein, mitochondrial genome region between gene Cox2 and gene Cox1 and Ras-related protein (Ypt1) gene of 12 *Phytophthora* isolates (six isolates from each group) were targeted. Similarity searches of assembled sequence data of the targeted genes using BLASTn programme showed wide variation among the members of the two groups of *P. capsici*.

Whole genome sequencing

De novo hybrid assemblies using sequence reads from two NGS platforms (Illumina and Roche/454) were made for two isolates of *Phytophthora*. The *de novo* hybrid assembly of two next-generation sequencing (NGS) technologies (Illumina and Roche/454 sequencing) yielded 63.8 Mb genome size at an N50 contig length of 4724 kb, with contig length ranging from 200- 42775 for smallest and largest contigs, respectively. The *de novo* hybrid assembly gave out 32044 contigs and 47280344 bases using Newbler Assembler. A reference assembly was also conducted to compare *P. capsici* genome of joint genome institute and percentage identity was 95.35% with an average read depth of 50X. Structural annotation was carried out using *ab-initio* gene prediction methods and an approximate of 22,358 coding sequences and 54485 exons were obtained. Simple sequence repeats (SSR) analysis revealed that there are 1344 SSRs out of 32044 contig sequence analysed. Whole genome alignment and comparison with reference genome revealed 1,298,146 SNP sites; 917 genes were common with reference genome of *P. capsici* (JGI), and 5501 genes are unique in *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. The newly assembled genome of *P. capsici* was structurally and functionally annotated to curate all possible gene by gene information.

Comparative genomics

Conserved domain search to identify the protein families present in exonic regions of whole genome sequences of *Phytophthora* sp. infecting black pepper (05-06) were studied. Blast2GO, Fast Annotator, and GoAnna were used for classifying gene ontology and unigenes were obtained. After identification of homology based unigenes, all the protein translations of exon sequences were subjected to CDD finding using RPS-BLAST, to identify the domain present in the genome. There were 1440 unique domain super families present; CDD search and InterProScan identified a total of 1,57,325 domains in whole genome.

The copy number, localization and comparative analysis of domains responsible for pathogenesis in IISR 05-06 and 98-93 isolates of *Phytophthora* with already published ones were attempted, and around 52 effector domains related to *Phytophthora* pathogenesis were identified and are being studied. ITS region of 102 *Phytophthora* isolates was sequenced and 50 sequences were submitted in NCBI. 90 Piper RGC's clones isolated using degenerate approach were sequenced and 54 sequences were deposited in NCBI.

Detection of pathogens

A real-time PCR protocol was developed for the detection of *P. capsici*. The primers for the assay were designed based on the RAPD-SCAR region (Acc. FN298514.1). The assay was standardized using DNA isolated from *P. capsici* isolate (06-04) as the positive control and *P. nicotianae* isolate (02-21) as negative control along with water control. Successful amplification was obtained in the positive sample with a Ct value of 13.21. For the quantification of *Phytophthora* in samples, a standard curve was constructed using genomic DNA as the standard. The genomic DNA was diluted from 1–10⁻⁷ dilutions and subjected to real time PCR in triplicates. The assay was standardized for the detection of *P. capsici* in soil. Real time PCR protocols were also standardized for *Radopholus similis* detection using species-specific primers.

Disease management

Evaluation of *Trichoderma* isolates

Trichoderma isolates obtained from different geographical locations in the network project, phytofura were evaluated against *P. capsici* under pot culture conditions for growth promotion and disease suppression. Among the 15 isolates evaluated, PhytoFuRa10 was highly promising resulting in 83.0% disease control when compared to control having a disease incidence of 85.6%. Highest growth promotion was observed in the isolate PhytoFuRa3 and highest biomass production by the isolate PhytoFuRa10.

Evaluation of actinomycetes

Four different actinomycetes strains (Act 2, Act 5, Act 6, and Act 9) individually and in consortia mode (Act 2+5, Act 2+9, Act 5+9, Act 2+6, Act 5+6, Act 6+9, Act 2+5+9, Act 2+6+9, Act 2+6+9) were evaluated for growth promotion and against nematodes and *Phytophthora*. Growth parameters of the plants were promising in consortia containing Act 2+5, 5+9 and 2+9 (Fig. 14). Dehydrogenase activity was also higher in these treatments showing the higher microbial activity in these treatments (Fig. 15). The treatments also showed reduced infection by *R. similis* and no *Phytophthora* incidence was also noticed in these treatments when compared to control and other treatments. The potential actinomycetes belonged to *Kitasatospora setae* (Act 2), *Streptomyces* (Act 5) and *S. tauricus* (Act 9).

The four promising actinomycetes (Act 2, Act 5, Act 6 and Act 9) antagonistic to *P. capsici* were evaluated for nematicidal activity *in vitro* and Act 2 and Act 9 were promising exhibiting 89% and 50% mortality, respectively.

Evaluation of endophytic bacteria in the field

In the field trial endophytic bacteria and chemicals are integrated maximum growth was observed in *T. harzianum* + *P. chlamydosporia* followed by *Curtobacterium luteum* + metalaxyl and *Pseudomonas putida* + carbosulfan treatments. No incidence of *Phytophthora* was noticed during the period.



Fig. 14. Root development in promising consortia of actinomycetes (a) Act 2 + 5 (b) Act 2 + 9 (c) Act 5 + 9 (d) control

Evaluation of resistant lines and biocontrol agents in the field

Two lines moderately resistant to *P. capsici* (C 1090 and IISR Shakthi) and one line resistant to *Radopholus similis* (HP 39) were evaluated along with promising bioagents *viz.*, *T. harzianum* and *P. chlamydosporia* in comparison with a susceptible variety Sreekara. The resistant lines planted by incorporating biocontrol agents exhibited better growth and were free from nematode and *Phytophthora* infection.

Evaluation of new fungicide against *P. capsici*

New fungicide *viz.*, RIL-070/FI was evaluated at different concentrations from 10 to 500 ppm of the

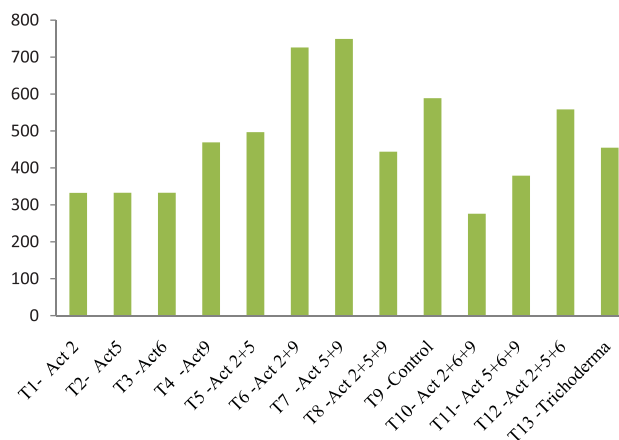


Fig.15. Dehydrogenase activity ($\mu\text{gTPF g}^{-1} \text{h}^{-1}$) in different treatments

product, *in vitro* against *P. capsici*. 100% mycelial inhibition was observed at 50 ppm and for inhibition of sporulation and zoospore germination, the maximum concentration required was 100 ppm and

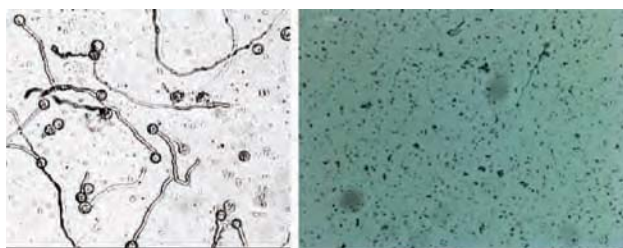


Fig. 16. Effect of RIL-070/FI on zoospore germination of *P. capsici*. (a) control (b) 200 ppm

200 ppm respectively. The average ED 50 and Ed 90 for *in vitro* inhibition was 29.23 and 54.43 ppm, respectively (Fig. 16, Table 2). Foliar spraying of the

Table 2. EC 50 and EC 90 values of RIL-070/FI for mycelial growth, sporangium production and zoospore germination of *P. capsici*

Growth phase	ED (µg/ml)	
	ED50	ED90
Mycelial growth	22.85	45.71
Sporangial production	34.47	47.47
Zoospore germination	30.38	70.11
Mean	29.23	54.43

Evaluation of chemicals against *R. similis*

Nematicidal activity of four chemicals *viz.*, fipronil, thiamethoxam, carbosulfan (G) and carbosulfan (EC) were evaluated against *R. similis* under pot conditions. Among the tested chemicals, fipronil (10 g) and carbosulfan (EC) (0.1%) caused

Table 3. Effect of soil application of RIL-070/FI on infection by *P. capsici*

Concentration (ppm)	Mortality (%)	Reduction in mortality over control (%)	<i>P. capsici</i> population	Reduction of <i>P. capsici</i> population (%)
200	14.3	75.0	36.06 (38.57)	46.0
300	14.3	75.0	19.29 (21.43)	71.1
400	0.00	100.0	14.97 (11.42)	77.6
600	0.00	100.0	14.97 (11.42)	77.6
Control	57.1	-	66.79 (74.43)	-
CD (P <0.05)			48.88 (54.54)	

chemical at concentrations from 100 to 600 ppm showed reduction in lesion development from 0.71% to 100%. When *P. capsici* was challenged inoculated five days after spraying of the chemical, 100% reduction in lesion development was observed at 600 ppm and the effect was reduced when challenge inoculated days after spraying (Table 3).

Evaluation of endophytic fungal metabolites on *R. similis*

Nine short listed endophytic fungal isolates which were effective against *P. capsici* were used for *in vitro* assay (metabolites) against *R. similis*. Maximum percentage mortality (60%) was observed by the isolate BPEF-73 (*Daldinia eschscholzii*), followed by 40% mortality by BPEF-75 (*Fusarium sp.*) (Fig. 17).

100% reduction in *R. similis* population (Fig. 18).

Evaluation of shelf life of *Pochonia chlamydosporia*

The survival of *Pochonia chlamydosporia* in 11 liquid formulations was evaluated at two temperatures (4°C and 25°C). Among the different formulations tested, liquid paraffin 5% could maintain the effective colony forming units (CFU) till 120 days at 25°C.

Viral diseases

Complete genome sequencing of PYMoV

Complete genome sequencing of *Piper yellow mottle virus* (PYMoV) from black pepper, betelvine and Indian long pepper was performed to obtain a better understanding of the genetic variability of the virus in different hosts. The overlapping fragments of

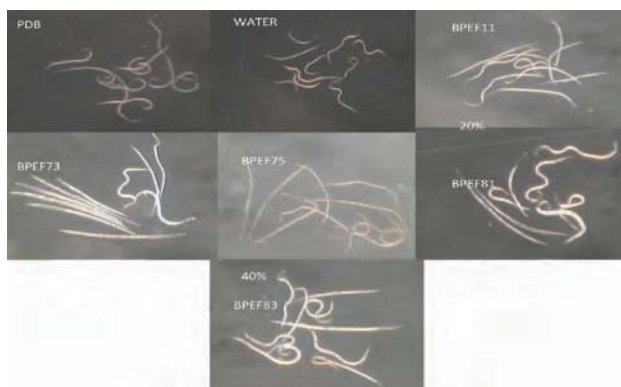


Fig. 17. Nematicidal activity of endophytic fungi

the genome amplified through PCR were cloned and their nucleotide sequence determined. The genome length of PYMoV isolates varied from 7549 to 7607 nucleotides in different hosts. The CLC genome viewer identified our open reading frames (ORFs) in all the three isolates of PYMoV. The open reading frame (ORF) III of the virus encodes for a polyprotein consisting of viral movement protein, trimeric dUTPase, zinc finger, aspartic protease, reverse transcriptase, and RNase H where as ORF I, II and IV encodes for proteins of unknown function. Whole genome sequence comparison showed an identity of 89-99% with one available PYMoV sequence while it

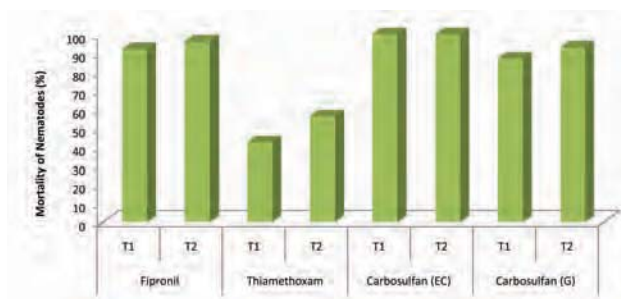


Fig. 18. Evaluation of chemicals against *R. similis*.

ranged from 39-56% with other badnavirus species indicating that badnavirus infecting black pepper, betelvine and Indian long pepper are strains of PYMoV. Nucleotide sequence of ORF I, II, III and intergenic region (IGR) and, amino acid sequence of ORF I, II and III showed significant identity between different PYMoV sequences ranging from 81-99%. 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 that were well separated from all other known badnaviruses. Among other badnaviruses, *Dioscorea bacilliform virus*, *Fig badnavirus 1*, *Cacao swollen shoot virus* and *Citrus yellow mosaic virus* were close to PYMoV isolates.

Genetic diversity of PYMoV

In order to understand the genetic variability of PYMoV, 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*. All isolates from black pepper, *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 with PYMoV indicating that they are variants of PYMoV. On the other hand high sequence variability of 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 the presence of new badnaviruses. Of these, virus isolates from *P. bababudani* and *P. chaba* showed an identity of 99% among each other suggesting that they belong to one badnavirus species. BLAST analysis of nucleotide sequence from *P. bababudani* and *P. chaba* showed high identity with PYMoV (77% - 79%) suggesting that they are most closely related to PYMoV. The virus isolates from *P. mullesua*, *P. peepuloides* and *P. thomsonii* indicated their distinctiveness as they shared only <71% identity in the nucleotide sequence between them. The BLAST analysis of nucleotide sequences from *P. mullesua*, *P. peepuloides* and *P. thomsonii* showed highest identity (68%) with different badnaviruses such as *Musa acuminata* endogenous badnavirus, *Taro bacilliform virus* and *Canna streak virus* respectively with sequence coverage up to 99% - 100% suggesting occurrence of new badnaviruses in each of these species.

Phylogenetic analysis of PYMoV isolates revealed that the 29 isolates formed three subgroups; subgroup

1 consisting of virus isolates from *P. attenuatum*, *P. barberi* and two isolates from *P. nigrum* (nigrum-17 and nigrum-8); subgroup 2 consisting 24 isolates and subgroup 3 consisting of only one isolate from *P. colubrinum*. The badnavirus isolates from *P. bababudani* and *P. chaba* showed very close relationship with PYMoV isolates while badnavirus isolates from *P. mullesua*, *P. peepuloides* and *P. thomsonii* showed very distant relationship with PYMoV. *Fig badnavirus 1* (FBV-1) was the closest badnavirus species to PYMoV isolates. Badnavirus isolate from *P. peepuloides* and *P. thomsonii* showed close phylogenetic relationship with *Taro bacilliform virus* (TaBV) while virus isolate from *P. mullesua* showed close relationship with *Pelargonium vein banding virus* (PVBV) and *Dracaena mottle virus* (DMV).

Influence of temperature on symptom expression of PYMoV

In the controlled experiment, symptomless PCR positive (indicating presence of PYMoV) and negative plants (indicating absence of PYMoV) were exposed to 35°C, 60% RH for duration of 8 h daily. Results showed that 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 virus copy number after exposure to temperature. Total chlorophyll and the total phenols in PCR negative plants was more than that of PCR positive plants initially while total proteins and total sugars were found to be higher after symptom expression. IAA content in PCR negative plants remained same before and after stress while the PCR positive plants showed higher content after the stress (5.11 µg g⁻¹).

Total proteins were extracted from leaves of PCR positive and PCR negative plants before and after exposure to heat. 2D Electrophoresis showed many unique up and down regulated proteins between control (PCR positive/PCR negative) and upon heat stress. Based on the spot volume criterion, a few spots were selected from low molecular (40-14KDa) and high molecular (60-70KDa) and were identified by nano LC-MS. The proteins identified from PCR negative after stress included calmodulin, class I heat shock proteins, rubisco large subunit, SOD and HSP

70. The proteins in PCR positive plants before stress were: photosystem i reaction center subunit ii (ferridoxin docking protein) and adenosyl homocysteinase while proteins in the PCR positive plants after stress included: subunits of oxygen evolving enhancer protein i, plastocyanin, mono dehydro ascorbate reductase, 2.cys peridoxin, chaperonin CPN 60-like protein, thylakoid luminal heat shock protein and CLP protease. The binding proteins included photosystem reaction center subunit I (ferridoxin docking protein) with RNaseH of virus; plastocyanin with RNaseH of virus and chaperonin CPN 60 protein with ORF III polyprotein of virus.

Anthracnose

Epidemiology

Studies on activation of microsclerotia of *Colletotrichum gloeosporioides* in runner shoots showed that the microsclerotia were activated within seven days when subjected to high humid conditions, by producing acervuli with setae and subsequent production of conidia embedded in a matrix under *in*

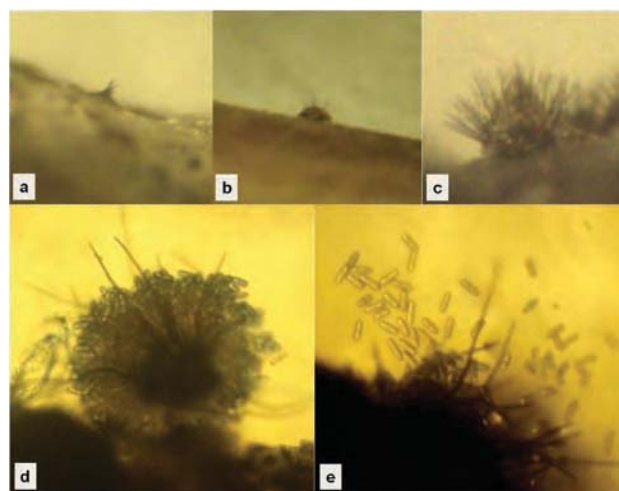


Fig. 19. Activation of microsclerotia of *C. gloeosporioides* (a-e) from black pepper tissues

vitro conditions (Fig. 19 a-e).

The incidence of foliar infection was characterized with grey necrotic lesions with black borders on older leaves of preceding season and unevenly distributed minute dark structures on the foliage. The dark structures produced orange coloured exudation, when incubated under high humid conditions. Microscopic observations revealed the presence of perithecia asci

and ascospores embedded in the exudates.

The leaf bit with exudate when inoculated on black pepper (var. Panniyur – 1), resulted in the formation of typical anthracnose symptoms. Subsequent isolation from the lesion yielded two distinct colonies, designated as black and orange. Pathogenicity 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.

Integrated disease management in the field

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb (0.1%), carbendazim (0.2%), Bordeaux mixture (1%) and hexaconazole (0.1%) and soil application of *T. harzianum*, singly and in combination showed that spraying carbendazim + mancozeb (0.1%) at 30 days intervals (three sprays) was superior over other treatments in reducing anthracnose incidence under field conditions (Table 4).

Table 4. Evaluation of fungicides and *Trichoderma harzianum* against anthracnose of black pepper under field conditions

Treatment	Per cent Disease Index	
	Initial	Final
Carbendazim + Mancozeb (0.1%)	45.83	16.67
Hexaconazole (0.1%)	37.50	41.67
Carbendazim (0.2%)	41.56	29.17
Bordeaux mixture (1%)	37.50	45.83
Carbendazim + Mancozeb + <i>T. harzianum</i>	41.56	20.33
Hexaconazole + <i>T. harzianum</i>	41.56	41.66
Carbendazim + <i>T. harzianum</i>	37.50	29.16
Bordeaux mixture + <i>T. harzianum</i>	37.50	45.83
<i>T. harzianum</i>	37.50	41.67
Untreated control	37.50	45.83
	CV (%)	10.75
	CD (0.05%)	6.72





CARDAMOM

Genetic resources

Around 592 accessions are being maintained in the National Active Germplasm Site which includes 442 accession of Appangala (CRC), 73 accessions of Pampadumpara (KAU), 47 accessions of Mudigere (ZHRS) & 30 accessions of Sakleshpur (ICRI)]. Five new accessions were collected from Megamalai, Tamil Nadu and added to the gene bank

Germplasm characterization

About 60 accessions have been characterized, Accession FGB-13 recorded maximum yield and

more number of capsules plant⁻¹ after one year of planting (Table 5)

Screening of germplasm

Natural incidence of leaf blight was recorded in 60 accessions maintained in the field gene bank at Appangala. The accessions were grouped into various categories based on the reaction towards leaf blight (Table 6).

Natural incidence of rhizome rot disease was recorded in 60 accessions maintained in the field gene bank at Appangala. The accessions were grouped into

Table 5. Mean, range and CV (%) for yield and yield attributes in cardamom germplasm

Character	Range	Mean	S.D	CV (%)	Promising genotype
Plant height (cm)	156-313	232.04	33.04	14.05	FGB 04
Bearing tillers	1.8-36.4	9.38	2.98	41.59	FGB 16
Leaf length(cm)	41.2-80.2	56.05	4.97	8.87	FGB 8
Leaf breadth (cm)	7.96-12.2	9.93	0.84	8.44	FGB 33
Total Panicles	2.4-40.3	11.54	6.01	52.05	FGB 32
No. of capsules	11.5-244.20	179.81	99.82	55.52	FGB 13
Wet weight (g)	11.25-399.61	160.93	101.81	63.26	FGB 13

Table 6. Reaction of field gene bank accessions against leaf blight

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

various categories based on the reaction towards rhizome rot disease (Table 7).

normalized. The nutrient contribution from soil in var. Green gold was quantified as 53% for N, 7.0% for P₂O₅

Table 7. Reaction of germplasm accessions against rhizome rot

Percent Disease Index	Category	Accessions
0.0 – 5.0	Highly resistant (HR)	FGB 1, FGB 3, FGB 8, FGB 9, FGB 13, FGB 21, FGB 22, FGB 26, FGB 28, FGB 29, FGB 30, FGB 45, FGB 52, FGB 60
5.1–10.0	Resistant (R)	FGB 5, FGB 19, FGB 27, FGB 31, FGB 33, FGB 34, FGB 49, FGB 50, FGB 58
10.1–25.0	Moderately susceptible (MS)	FGB2,FGB4,FGB6,FGB7,FGB10,FGB11,FGB12,FGB14,FGB15,FGB16,FGB17,FGB18,FGB20,FGB23,FGB25,FGB32,FGB35,FGB44,FGB46,FGB47,FGB48,FGB51,FGB53,FGB54,FGB55,FGB56,FGB57,FGB59
25.1–50.0	Susceptible (S)	FGB 24, FGB 36, FGB 37, FGB 38, FGB 41, FGB 42, FGB 43
> 50	Highly susceptible (HS)	FGB 39, FGB 40

Screening of germplasm for resistance to cardamom thrips

Screening of germplasm at CRC, Appangala for identification of sources of resistance to thrips (*Sciothrips cardamomi*) was continued for the third consecutive year in association with Indian Institute of Horticultural Research, Bangalore. About 278 accessions were screened during the year. One accession recorded total capsule damage below 10%. 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 > 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 hybrids under Co-ordinated varietal trial (CVT)

Four genotypes [IC 547167 (NH_y 35); IC 349651 (AMB 2); IC 349545 (M 28); IC 547185 (VA 1)] are identified as high yielding based on the three year yield evaluation under coordinated varietal trial at Appangala (Table 8, Fig. 20).

Quantifying the nutrient removal for target yield

Based on pooled yield and nutrient uptake data the constant parameters i.e. nutrient contribution from soil (CS), fertilizers (CF) and Nutrient requirement for production of 100 kg of produce (NR) were



Fig 20. Promising cardamom genotypes

Table 8. Yield performance of promising genotypes

Accession	Yield kg/ha			Mean
	2011-12	2012-13	2013-14	
IC 349545	1085.33	957.87	625.48	889.56
IC 349651	1764.00	801.25	525.98	1030.41
IC 547167	1393.12	716.80	671.94	927.29
IC 547185	1449.60	870.34	498.59	939.51
CL 691	606.30	380.34	354.73	447.12
CL 726	593.93	321.46	301.45	405.61
PL NO. 14	1042.92	648.67	539.04	743.54
CR 6	419.50	297.43	255.76	324.23
MCC 346	521.55	461.36	327.97	436.96
SKP 104	718.10	398.67	327.73	481.55
SKP 164	852.60	423.35	349.21	41.72
Mean	949.72	570.69	434.35	651.59
SD	436.88	235.91	142.23	
CV%	46.00	41.34	32.75	
CD(P<0.05)	391.23	280.04	174.39	

and 9.9% for K_2O and in var. Appangala-1 as 37% for N, 17.5% for P_2O_5 and 19.1% for K_2O . The nutrient contribution from fertilizer was worked out to be 61% for N, 9.6% for P_2O_5 and 38.7% for K_2O in Green gold and 48% for N, 7.6% for P_2O_5 and 26.6% for K_2O in Appangala-1. The fertilizer recommendation equations for varying soil test values and yield targets were developed.

Drought tolerance studies

Twelve short listed cardamom genotypes along with three checks were evaluated in control and stress treatments. Moisture stress was imposed by withholding irrigation during fourth year. Growth, yield and physiological parameters (relative water content and specific leaf weight) were recorded in control and stress treatments. Soil moisture content ranged from 12-17% under stress.

Relative water content was determined in control and stress treatment. Stress was imposed by keeping leaf at 45°C for three hours in BOD incubator. Percent relative water content reduction over control was calculated. It ranged from 10.77 (APG 224) to 35.72 % (IC 584071) with a mean of 22.35. Specific leaf weight $mg\ cm^{-2}$ ranged from 5.45 (IC 584058) to 7.33 (NKE19 x GG) with a mean 6.15 $mg\ cm^{-2}$. Genotypes, IC 584070, IC 584071, APG 224, GG and NKE 19 x GG with larger leaves took longer time (6h) to fold compared to Appangala 1.

Growth and yield parameters were generally reduced under stress. Total number of tillers per clump ranged from 16.46 (IC 584059) to 40.7 (Appangala 1) with a mean of 22.31 in control and in stress it ranged from 9.93 (NKE 19 x GG) to 15.8 (Appangala 1) with a mean of 13.

E-nose development

In order to select a suitable sensor array for determining the quality of cardamom, Normaroma index was determined using an integrated Electronic–Nose–Vision system connected with three sensor arrays. The three sensor arrays tested included the sensor array used for determination of quality of tea, sensor array for waste-water analysis, and a 6-sensor array. The chief components of cardamom oil, namely, 1,8-cineole and α -terpinyl acetate were mixed

in different proportions and Normaroma index was determined using the above three sensor arrays. The response from each sensor in the array was decoded and analyzed statistically. Based on the results a new sensor array was designed using the sensors with high standard deviation.

Rhizome-root rot

Etiology

Inoculation studies under glass house conditions with *Pythium vexans*, *Rhizoctonia solani* and *Fusarium oxysporum* individually and in combinations on seedlings (var. Appangala-1) indicated that inoculation with *P. vexans* alone resulted in 66.7 % mortality. Sequential inoculation of *P. vexans* followed by *R. solani* recorded 83.3% mortality of the seedlings. Observations on sequential inoculation studies on cardamom seedlings indicated that *P. vexans* and *R. solani* were primarily involved in rhizome rot infection and *F. oxysporum* caused root rot damage to seedlings.

Studies on sequential events in the colonization and proliferation of *P. vexans*, *R. solani* and *F. oxysporum* showed that *P. vexans* required only 4h to colonize the

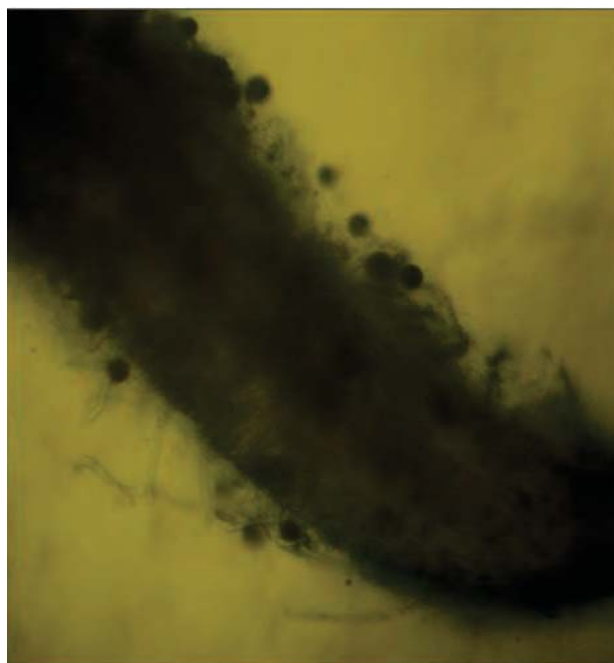


Fig. 21. Production and aggregation of sporangia of *P. vexans* near the root tip region of cardamom

roots, whereas *R. solani* and *F. oxysporum* required 12 and 96h, respectively. Under high humid conditions, sporangia of *P. vexans* were produced in abundance and aggregated near the root tip region (Fig. 21). The

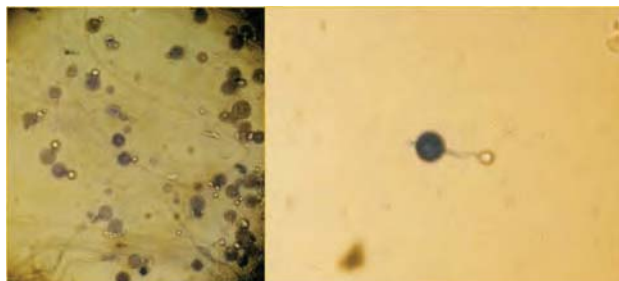


Fig. 22. Germination of sporangia (a) directly by germ tubes or (b) indirectly, through formation of vesicles containing zoospores

sporangia germinated either directly by germ tubes or indirectly, through the formation of vesicles containing zoospores (Fig. 22). *R. solani* initially

produced primary and secondary hyphal branches and later several side branches formed were modified into infection structures like bulbous and lobate appressoria. Whereas, *F. oxysporum* colonized the root surface with thin hyphae forming a dense mat of mycelium covering the root surface 96h after inoculation.

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* (21.4% – 67.8%), *R. solani* (44.4% – 60.7%) and *F. oxysporum* (49.6%–77.4%).

Screening of chemicals

Among the seven fungicides tested against *P. vexans* under *in vitro* conditions, fenamidone +

Table 9. Mycelial compatibility among *Colletotrichum* isolates from cardamom - black pepper cropping system

Isolates	Cardamom Malabar	Vazhukka (streak symptom)	Vazhukka (spot symptom)	Coffee (Liberian)	Cocoa	Colocasia	Turmeric	Cassia	Cardamom-Mysore	Clove	Coffee (Arabica)	Black Pepper	Black Pepper Wild	Coffee (Robusta)	Dadap	Silky Oak	Bird's eye chilli	Mango	Nutmeg	Avocado
Cardamom																				
Malabar	C	IC	IC	IC	IC	IC	IC	IC	C	C	C	C	C	C	C	C	C	IC	C	IC
Vazhukka (streak symptom)		C	C	C	C	C	IC	C	C	C	C	C	IC	IC	IC	C	C	IC	C	IC
Vazhukka (spot symptom)			IC	C	IC	C	IC	C	C	C	C	IC	IC	IC	IC	C	C	C	IC	IC
Coffee (Liberian)				C	C	C	C	C	C	C	C	IC	IC	C	IC	C	C	IC	IC	IC
Cocoa					C	IC	IC	IC	C	C	C	IC	C	IC	C	C	C	IC	IC	C
Colocasia						C	C	IC	C	IC	IC	IC	C	C	IC	C	IC	C	IC	C
Turmeric							C	C	C	IC	C	IC	C	IC	IC	IC	C	IC	IC	C
Cassia								C	C	C	IC	IC	IC	C	C	C	C	C	C	IC
Cardamom-Mysore									C	IC	C	C	IC	C	IC	IC	C	C	C	IC
Clove										C	C	C	C	IC	C	C	C	C	IC	C
Coffee (Arabica)											C	IC	IC	C	IC	C	IC	C	IC	C
Black Pepper												C	IC	C	IC	C	C	IC	IC	IC
Black Pepper Wild													C	IC	C	IC	C	IC	IC	C
Coffee (Robusta)														C	IC	C	IC	IC	C	IC
Dadap															C	C	IC	IC	C	IC
Silky Oak																C	C	IC	IC	IC
Bird's eye chilli																	C	C	C	C
Mango																		C	C	C
Nutmeg																			C	C
Avocado																				C

C-Compatible : IC-Incompatible



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 galanga* (2 collections), *Alpinia mutica*, *Zingiber zerumbet*, *Amomum subulatum*, *Aframomum melegeuta*, *Amomum* sp., and *Hedychium coronarium* 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) had

Leafspot

Mycelial compatibility among the isolates of *Colletotrichum*

Studies on mycelial compatibility among isolates of *Colletotrichum* from cardamom - black pepper cropping systems showed that the isolates from Liberian coffee (*Coffea liberica*), clove and cardamom (*Malabar* ecotype) were more compatible with other isolates. But the isolates from silky oak, avocado, Robusta coffee, dadap and mango were less compatible with other isolates tested (Table 9).

Integrated disease management in the nursery

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb (0.1%), carbendazim (0.2%) and Bordeaux mixture (1%), 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

Table 10. Evaluation of fungicides and *Trichoderma harzianum* against leaf spot disease of cardamom in the nursery

Treatment	Initial	Per Cent Disease Index	
		Final (Spray interval-15 days)	Final (Spray interval-30 days)
Carbendazim + Mancozeb (0.1%)	45.77	13.43	20.00
Carbendazim (0.2%)	50.92	20.56	31.62
Bordeaux mixture (1%)	49.99	26.11	26.11
Carbendazim + Mancozeb + <i>T. harzianum</i>	54.95	14.02	32.04
Carbendazim + <i>T. harzianum</i>	51.38	23.33	32.76
Bordeaux mixture + <i>T. harzianum</i>	51.54	34.44	26.44
<i>T. harzianum</i>	43.67	34.60	34.60
Untreated control	50.25	55.32	55.32
	CV (%)	14.39	11.26
	CD (0.05)	7.85	6.79

inhibitory effect on the growth of *C. gloeosporioides* (53.7 %). Among the endophytes isolated from *Malabar* ecotype, the isolates *viz.*, MA 1, MA 3, MA 4, MA 7, MA 9, MA 10, MA 11 and MA 13 were found to have superior antagonistic activity against *Colletotrichum gloeosporioides* under *in vitro* conditions, compared with other isolates. Whereas, among the endophytes isolated from *Mysore* ecotype, the fungal isolates *viz.*, MS 1, MS 2, MS 3, MS 12 and MS 13 were found superior under *in vitro* conditions.

leaf spot incidence of cardamom under nursery conditions (Table 10).

Integrated disease management in the field

Validation of efficacy of fungicides *viz.*, carbendazim + mancozeb (0.1%), carbendazim (0.2%) and hexaconazole (0.1%), and soil application of *T. harzianum*, singly and in combination showed that combined application of hexaconazole (0.1%) (3 sprays) and soil application of *T. harzianum* at 30 days interval (three applications) was promising in reducing the incidence of leaf blight in the field

Table 11. Evaluation of fungicides and *Trichoderma harzianum* against leaf blight of cardamom in the field

Treatment	Percent Disease Index		
	Initial	Final (Spray interval - 15 days	Final (Spray interval - 30 days)
Carbendazim + Mancozeb (0.1%)	53.62	25.28	21.36
Hexaconazole (0.1%)	53.96	17.79	18.09
Carbendazim (0.2%)	50.69	20.93	18.88
Carbendazim + Mancozeb + <i>T. harzianum</i>	59.65	38.42	20.10
Hexaconazole + <i>T. harzianum</i>	54.96	17.74	17.85
Carbendazim + <i>T. harzianum</i>	57.92	25.44	18.98
<i>T. harzianum</i>	55.55	46.29	46.29
Untreated control	73.33	61.38	61.38
	CV (%)	11.93	27.07
	CD (0.05)	6.66	13.33

(Table11).

Cardamom thrips

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 thrips in association with Indian Institute of Horticultural Research, 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), spinosad (0.3 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), thiamethoxam (0.3 mL L⁻¹) and spinosad (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 cardamom thrips populations varied from 15.0-87.8 % in various areas in Kerala, Karnataka and Tamil Nadu. The mean infection rate was 53.5% with 57.1% male and 50.6% female populations infected by the bacterium. 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 used in the study from cardamom thrips collected from different areas clustered together showing 99% similarity among them indicating that irrespective of geographical isolation, all the thrips were infected by the same *Wolbachia* strain, *wScar*.

Studies on entomopathogens

The entomopathogenic fungus isolated from cadavers of cardamom thrips from Wayanad District was identified as *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales) (Fig. 23). 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 × 10⁷ conidia mL⁻¹), up to 62.9% mortality was recorded in the test population, 10 days post

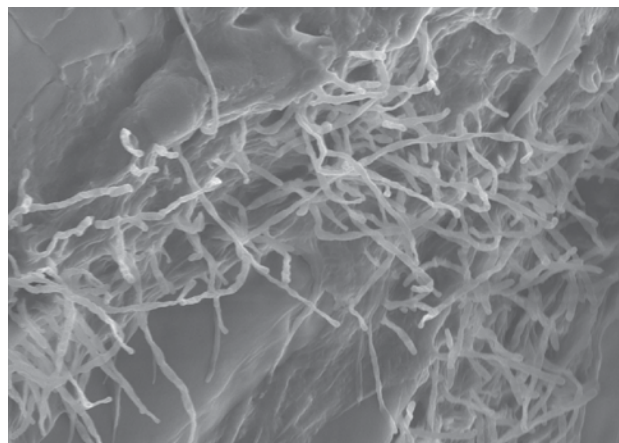


Fig. 23. Mycelial growth of *Lecanicillium psalliotae* on cardamom thrips

inoculation. The ITS rDNA, partial β -tubulin and partial translation elongation factor 1 α 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 a first report of a fungus infecting cardamom thrips. The isolate is maintained in the Entomopathogenic Fungi

Repository of IISR as IISR-EPF-02.

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



GINGER

Genetic resources

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

Yield evaluation of promising accessions

Extra bold types

Based on visual selection for boldness, 13 lines were selected for its extra bold rhizome character

from the germplasm and evaluated for yield. The different accessions varied in plant morphological characteristics. Among the 13 accessions studied, maximum yield was recorded in Acc. 723, Acc. 247 followed by Acc. 713 (Table 12).

Low fiber types

Seven promising low fibre accessions of were evaluated for yield. Among the accessions studied maximum yield was recorded in Acc. 278 followed by IISR Varada (Table 13).

Table 12. Yield and yield attributes of bold type ginger

Genotype	Plant height (cm)	No. of tillers	No. of leaves tiller ⁻¹	No. of leaves plant ⁻¹	Leaf length (cm)	Leaf width (mm)	Yield bed ⁻¹ (3m ²)	Projected yield ha ⁻¹ (t)
727	53.87	9.20	15.47	87.40	20.50	27.47	4.35	10.87
714	46.67	12.07	11.20	71.67	19.70	25.17	4.81	12.03
287	41.93	9.60	11.93	75.33	18.87	25.47	4.75	11.89
397	42.87	10.87	12.53	90.80	19.07	26.40	5.00	12.50
249	49.47	8.20	13.00	65.07	21.03	26.13	3.91	9.77
689	56.53	11.13	14.60	63.40	21.57	26.17	3.88	9.69
713	48.00	11.00	13.40	70.07	18.87	26.03	9.98	24.95
247	58.40	8.00	16.00	76.47	20.67	26.90	10.09	25.21
723	54.53	9.07	12.53	63.67	21.43	26.53	11.12	27.81
726	47.20	8.87	12.13	61.13	18.23	24.40	6.72	16.80
702	51.13	13.00	12.87	87.93	21.07	24.33	6.52	16.31
821	49.00	14.07	15.20	81.87	19.77	26.03	3.21	8.03
701	50.33	11.00	14.73	89.53	21.93	25.70	7.28	18.19
IISR Varada	50.40	11.23	15.30	83.11	23.17	28.07	9.11	22.78
Mean	50.02	10.52	13.64	76.25	20.42	26.06	6.48	16.20
CV (%)	10.25	27.96	18.37	25.68	8.47	8.74	11.86	
CD (0.01)	8.59	NS	NS	NS	NS	NS	1.69	



Table 13. Yield and yield attributes of low fiber ginger

Genotype	Plant height (cm)	No. of tillers	No. of leaves tiller ⁻¹	No. of leaves plant ⁻¹	Leaf length (cm)	Leaf width (mm)	Yield bed ⁻¹ (3m ²)	Projected yield ha ⁻¹ (t)
Acc. 282	61.33	16.07	17.27	122.73	19.60	25.67	7.36	18.40
Acc. 272	56.73	12.67	14.53	90.73	21.13	26.33	6.84	17.11
Acc. 82	62.27	9.67	18.27	99.53	23.30	26.53	6.12	15.29
Acc. 91	57.47	14.00	17.40	76.73	23.80	30.30	8.33	20.83
Acc. 278	48.53	12.47	11.60	97.73	19.80	25.07	9.48	23.71
Acc. 87	59.80	10.47	12.40	60.93	21.47	27.80	6.77	16.93
Acc. 239	60.87	5.33	18.33	57.67	20.60	26.70	8.71	21.78
IISR Varada	73.27	11.93	20.33	127.80	25.17	29.30	9.31	23.28
Mean	60.03	11.58	16.27	91.73	21.86	27.21	7.87	19.67
CV (%)	3.32	11.32	7.30	24.52	4.72	3.04	10.60	
CD (0.01)	3.99	2.57	2.38	45.04	2.07	1.66	1.67	

High oil types

A trial is laid out with 8 high oil accessions along with check, IISR Varada. Morphological and yield characters were recorded. Among the eight accessions, mean yield per bed (kg 3m²) ranged from 3.73-8.10. Maximum yield was recorded in Acc. 411, followed by Acc. 420.



Fig. 24. Screening of V1M1 mutants against *Ralstonia solanacearum*

Mutation induction

Four thousand one hundred and twenty rhizome buds were subjected to gamma irradiation at different doses of 0.80, 0.90 and 1.00 kR at KAU, Thrissur,

Kerala. 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 identifying three mutants without infection (Fig. 24). These mutants will be multiplied and subjected to secondary screening.

Four mutants that escaped three rounds of *Ralstonia solanacearum* infection were clonally multiplied to take up further yield evaluation.

Host-defense strategies - Comparison of transcriptomes of ginger and mango ginger

To determine the effect of the infection by the *R. solanacearum* on gene expression in *C. amada* and *Z. officinale*, both the transcriptomes were compared. A total of 20,938 *C. amada* and 20,061 *Z. officinale* genes were expressed. The differential expression analysis was performed using either RPKM or count data. Based on 3-fold change and FDR P value <0.005 total 1201 gene have been identified as differentially expressed, out of which 587 genes are upregulated and 613 genes are down regulated. The up regulated genes were further classified into functional categories related to defense response, pathways and molecular function with respect to bacterial infection. A singular enrichment analysis of GO terms also revealed that

defense related GO terms are significantly enriched at $P < 0.005$. Among the 54 differentially expressed transcription factors, 34 are up regulated in *C. amada* which includes WRKY, MYB, leucine zipper protein, zinc finger and GATA domain transcription factors (Table 14).

Table 14. Summary of transcription factor unigenes of *C. amada* and *Z. officinale*

Transcription factors family	Number of genes detected	Up-regulated in <i>C. amada</i>	Up-regulated in <i>Z. officinale</i>
WRKY	8	4	4
MYB	6	4	2
AP2/ERF	2	2	-
MYC	1	1	-
GRAS	1	1	8
Zinc finger	17	9	1
bHLH	1	-	1
bZIP	3	2	4
Others	15	11	16
Total	54	34	20

Genes involved in mevalonate pathway (MEP) for biosynthesis of isoprene/terpenes were upregulated substantially in *C. amada* compared to *Z. officinale* (Table 15).

Table 15. List of upregulated isoprene/terpene biosynthesis genes in *C. amada*

Protein name	Fold change	GO Functions
Fructose-bisphosphate aldolase (EC 4.1.2.13)	3901.00	GO:0006098 pentose-phosphate shunt GO:0015976 carbon utilization
1-D-deoxyxylulose 5-phosphate synthase	10.36	GO:0016114 terpenoid biosynthetic process GO:0006694 steroid biosynthetic process
4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	34.25	GO:0006694 steroid biosynthetic process
1-deoxy-D-xylulose 5-phosphate reductoisomerase	17.50	GO:0016114 terpenoid biosynthetic process GO:0019288 isopentenyl diphosphate biosynthetic process, mevalonate-independent pathway
4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	27.90	GO:0009862 systemic acquired resistance, salicylic acid mediated signaling pathway GO:0019288 isopentenyl diphosphate biosynthetic process, mevalonate-independent pathway GO:0009617 response to bacterium
Terpene synthase activity	3.03	GO:0000287 magnesium ion binding GO:0010333 terpene synthase activity
2-C-methyl-D-erythritol 2, 4-cyclodiphosphate synthase	1.87	GO:0016114 terpenoid biosynthetic process
1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase	2.55	GO:0016114 terpenoid biosynthetic process GO:0019288 isopentenyl diphosphate biosynthetic process, mevalonate-independent pathway

Organic farming

Ginger was grown by applying standardized organic package in comparison with the integrated and chemical systems to test their sustainability. In soil, phosphobacteria, azospirillum and pseudomonas population did not show any significant difference among all varieties under organic treatment. Under chemical system, microbial population was low compared to the other two systems. Acid phosphatase activity was higher under organic on par with that of integrated management, while the chemical

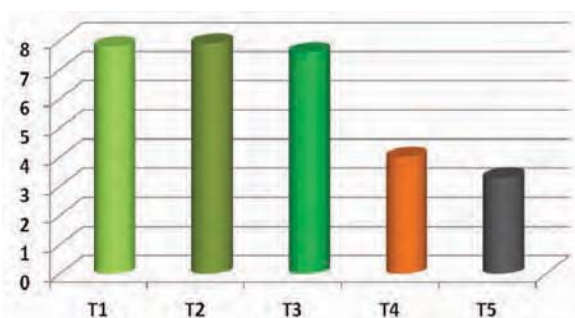


Fig. 25. Effect of PGPR delivery systems on ginger yield (kg 3m² bed)

management system recorded the lowest. Alkaline phosphatase, phosphodiesterase and dehydrogenase activities were significantly higher under organic system. Soil available P and K contents were higher under inorganic and integrated systems and that of OC, Ca, Mg and Zn were higher under organic system. Higher yield (8.5-12.3 kg 3m⁻²) was recorded under organic management system followed by integrated (6.4-8.5 kg 3m⁻²) and inorganic (6.3-6.7 kg 3m⁻²) management systems.



Fig 26. IISR GRB35 (*B. amyloliquefaciens*) in gelatin capsules

Validation of encapsulation technique for delivery of PGPR

While encapsulating techniques have been fairly successful in the laboratory, attempts to emulate the performance in the field have been largely unsuccessful. Therefore, presently no such commercial products are available in the market. However, we have successfully encapsulated and field tested the delivery of a plant growth promoting rhizobacteria (IISR GRB35- *Bacillus amyloliquefaciens*) for growth promotion and disease control in ginger. Field experiment conducted during 2013-14 to test the efficiency of this novel PGPR delivery using IISR GRB 35 in biocapsules revealed that application of GRB 35 cell suspension (T1), one capsules 5 kg⁻¹ seed (T2) and two capsules 5 kg⁻¹ seed

(T3) registered comparable yields (7.9, 7.6 and 7.8 kg 3 m⁻², respectively; Fig. 25). However, these yields were significantly greater than Metalaxyl-mancozeb-T4 (4.0 kg 3m⁻²) and absolute control-T5 (3.3 kg 3m⁻²). The study revealed the remarkable efficiency of delivering PGPR through biocapsules. Patent for this delivery process has been filed (Fig. 26).

Effect of weed management practices on growth, yield and quality

An experiment was conducted to study the effect of weed management practices having combination of different leaf compost mulches and its doses on weed control efficiency and on growth, yield, quality parameters and disease incidence. The major weed species found in the ginger field were dicots like *Neanotis tubulosa*, *Ficus hispida*, *Physalis angulata*, *Syndrella nodiflora*, *Crassocephalum crepidioides*, *Ipomoea marginata*, *Achyranthes aspera*, *Celosia argentea*, *Brachiaria ramosa*, and monocotyledons like *Cyanotis spp* and *Cyperus digitatus*. In total 51 weed species were identified.

Maximum plant height (43.22 cm plant⁻¹) and tiller production (5.55 plant⁻¹) were recorded in the treatment with application of paddy straw as mulch at the time of planting which was on par with the application of coir pith compost and coconut leaves with green leaf mulches at 45 and 90 DAP. At 45 DAP, significantly lesser weed dry matter (168.75g) and higher weed control efficiency was recorded in the treatment with paddy straw which was on par with coir pith compost and coconut leaves. Soft rot incidence was less in plots mulched with coconut leaves alone (8.70%) followed by incorporation of cowpea leaves. Higher yield (13.03 t ha⁻¹) was also recorded in the treatment where paddy straw was mulched.

Transplanting of single sprout seedling

A replicated trial with four treatments was laid out to standardize the transplanting single bud sprout technique (Fig. 27). The results revealed that there was 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 cost.



Fig. 27. Pro-trays raised ginger seedlings from single bud ready for transplanting

Evaluation of antagonists

Four new isolates of phages were isolated from ginger rhizosphere and evaluated against *R. solanacearum* biovar 3. No infection in the plant after challenge inoculation was observed showing the biocontrol potential of the phage. The *in vitro* inhibition is manifested as clear zones which ranged from 2-6mm. A total of 150 bacteria were isolated from the apoplastic fluid of pseudostem and leaves of collected from different areas and different ginger accessions. These were evaluated *in vitro* and *in planta* against *R. solanacearum* biovar 3 for biocontrol potential and isolates, IISR GAB 24, IISR GAB 42, IISR GAB 43, IISR GAB 48 IISR GAB 107, and IISR

Table 16. Details of *R. solanacearum* isolates collected from Kerala and Karnataka

Isolate name	Host plant	Place of collection	Pathogenicity on ginger	Days taken for wilting	Biovar
GRs Mep 2	Ginger	Meppadi, Wayanad	+	23	3
GRs Mep 3	Ginger	Meppadi, Wayanad	+	08	3
GRs Mep 4	Ginger	Meppadi, Wayanad	+	07	3
CaRs Mep 3	Small cardamom	Meppadi, Wayanad	+	07	3
GRs Mnt 5	Ginger	Manathavadi, Wayanad	+	11	3
GRs Mnt 6	Ginger	Manathavadi, Wayanad	+	12	3
GRs Mnt 7	Ginger	Manathavadi, Wayanad	+	11	3
GRs Idk 1	Ginger	Adimali, Idukki	+	07	3
GRs Idk 2	Ginger	Kumali, Idukki	+	17	3
GRs Spr	Ginger	Kerodi, Sakleshpur	+	09	3
TRs Klm	Tomato	Kayamkulam, Kollam	-	No wilting	3
GRs And	Ginger	Andoor, Wayanad	+	11	3
GRs Pul 3	Ginger	Pulpally, Wayanad	+	12	3
GRs Sik	Ginger	Sikkim	+	07	3
GRs Mnt	Ginger	Manathavadi, Wayanad	+	15	3
GRs Mnt 2	Ginger	Manathavadi, Wayanad	+	10	3
GRs Pkd	Ginger	Palakkad	+	14	3
GRs Tms 2	Ginger	Thamarassery, Kozhikode	+	17	3
GRs Tly	Ginger	Thirunelli, Wayanad	+	08	3
CaRs Mep	Cardamom	Meppadi, Wayanad	+	08	3

Bacterial Wilt

Collection of isolates of *Ralstonia* and cross infectivity studies

Twelve new isolates of *R. solanacearum* were collected from different ginger growing areas of Kerala and Karnataka; one isolate was also collected from cardamom. The isolates were characterized for biovar, pathogenicity and virulence. Pathogenicity of all the isolates was confirmed by inoculation on respective host plants (Table 16).

GAB 146, were short listed as effective in suppressing bacterial wilt in ginger.

Shoot borer

Evaluation of EPNs

The infectivity of four promising EPNs viz., *Heterorhabditids* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius gingeri* (IISR-EPN 07) and *Oscheius* sp. (IISR-EPN 08) were 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 during 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 on par with malathion 0.1% treatment (17.4% and 25.3%, respectively).

Compatibility of EPNs with pesticides

The effect of pesticides such as malathion 0.1%, chloropyrifos 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 chloropyrifos; however, mancozeb adversely affected the activity of *Heterorhabditids* sp. (IISR-EPN 01), *O. gingeri* and *Oscheius* sp. (IISR-EPN 08) (34% to 57% mortality, respectively).

Documentation of natural enemies of spice crop pests

Surveys were conducted in over 78 locations in nine districts in Kerala, Karnataka and Tamil Nadu to document entomopathogens and other natural enemies of insect pests of spice crops (Table 17).

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 *Protopulvinaria* sp.) and cardamom (*Aulacaspis* sp.). Three larval and three pupal parasitoids belonging to Braconidae, Ichneumonidae and Tachinidae were recorded on shoot borer infesting ginger and cardamom. A nucleopolyhedrosis virus was recorded from *Udaspus folus* (IISR-NPV-01) and *Spilosoma* sp. (IISR-NPV-02) infesting turmeric. Coleopteran predators such as *Chilocorus circumdatus* and *C. nigritus* were recorded on mussel scale infesting black pepper. The entomopathogens isolated from spice crop pests are maintained in the Institute biocontrol repository as IISR-EPF-03 to IISR-EPF-11 (*Isaria* sp., *Paecilomyces* sp. and *Lecanicillium* sp.).

Table 17. Survey locations for natural enemies of spice crop pests

State	District	No. of locations	Crops
Karnataka	Kodagu	17	Cardamom, Black pepper, Ginger, Nutmeg
	Chamarajanagar	02	Turmeric
	Chikkamagaluru	05	Cardamom, Black pepper, Ginger
	Shimoga	02	Black pepper, Ginger
Kerala	Wayanad	20	Cardamom, Black pepper, Ginger
	Kozhikode	08	Black pepper, Ginger, Turmeric, nutmeg, Cinnamom
Tamil Nadu	Coimbatore	05	Turmeric
	Erode	11	Turmeric, Ginger
	Namakkal	08	Black pepper, Turmeric



TURMERIC

Genetic Resources

One thousand four hundred and four *Curcuma* accessions are being maintained in the field gene bank. Germplasm conservatory was enriched with a six new accessions including a unique *C. amada* accession from Andhra Pradesh with purple pigmentation in midrib. Two hundred and forty seven first generation seedling progenies are also being maintained.

Yield evaluation of germplasm selections

A multilocal trial with three promising turmeric accessions (Acc. 48, Acc. 79 and Acc. 849) along IISR Prathiba and local check were laid out in Kerala (Peruvannamuzhi), Andhra Pradesh (Vijayawada), Tamil Nadu (Erode) and Karnataka (Appangala). The maximum dry yield across locations was recorded in Acc. 849, followed by Salem Local and Acc. 48 (Fig. 28). The highest curcumin was recorded in IISR Prathiba followed by Acc. 48 (Fig. 29).

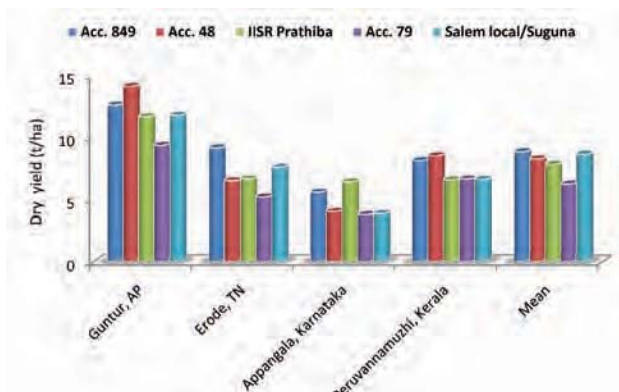


Fig. 28. Yield status of turmeric entries in multilocal trial

Yield evaluation of true seedling progenies

Twenty four first generation seedlings and a soma-



Fig. 29. Promising high yielding turmeric accession

clone were planted in the field in three replications with five released varieties as control at IISR Experimental Farm, Peruvannamuzhi. Highest yield of 9.43 Kg 3 m² was produced by control Kedaram followed by 9.23 Kg 3 m² by a somaclone SC61. Among seedlings highest yield was produced by 138/32 (8.27 Kg 3 m²) (Table 18).

Table 18. Yield of seedling progenies

Genotype	Yield (Kg 3 m ²)
18/11	4.60 ij
18/12	5.10 hij
18/13	7.67 abcdef
20/7	7.50 abcdefg
65/12	7.77 abcdef
69/5	6.87 cdefghi
69/10	7.07 bcdefgh
126/1	6.87 cdefghi
126/5	7.00 bcdefgh
138/20	6.77 cdefghi
138/24	5.17 ghij
138/30	6.23 cdefghij
138/32	8.27 abcd
138/43	6.17 cdefghij
138/46	6.07 defghij
138/48	6.97 bcdefgh
138/51	7.07 bcdefgh
138/78	5.83 efghij
389/1	7.83 abcdef
414/3	8.17 abcde
415/3	5.70 fghij
415/9	6.77 cdefghi
449/3	6.27 cdefghij
449/6	6.97 bcdefghi
SC61	9.23 ab
Rajendra Sonia	8.47 abc
Sudarsana	8.20 abcde
Suvarna	7.97 abcdef
Prabha	4.40 j
Kedaram	9.43 a

Means followed by the same letter are not significantly different at $\alpha=0.05$ of DMRTLSD at $\alpha=0.05=1.931$



Nine first generation seedlings are evaluated at CRC, Appangala with released variety IISR Prathiba as control. Highest yield was produced by control Prathiba 12.28 Kg 3 m². Among seedlings 449/6 produced the highest yield of 7.53 Kg 3 m².

Quality analysis of the processed rhizomes from the replicated trial 2012 (2013 harvest) was performed. The seedling progeny 389/1 continued to give >5% curcumin content (Table 19). At Peruvannamuzhi it was 5.77%, while at Appangala it was 5.26%.

Table 19. Yield and quality of shortlisted seedling progenies of turmeric

Identity	Dry Recovery (%)	Curcumin (%)	Oil (%)	Oleoresin (%)
126/1	18.02	3.14	4.27	10.33
126/5	19.17	3.58	4.44	11.60
138/22	19.28	3.05	4.43	11.79
138/32	19.67	2.58	4.10	8.95
138/43	23.17	1.12	5.20	10.73
138/78	19.90	3.42	5.73	13.25
389/1	18.82	5.26	4.77	15.04
415/3	18.40	2.25	4.50	10.91
449/6	18.83	3.98	4.30	12.43
IISR Prathiba	18.28	4.94	3.20	9.92
LSD at $\alpha=0.05$	1.46	0.50	0.63	1.48

Reproductive biology and cytology

Seffling and crossing studies were initiated with

high curcumin line 389/1, second generation seedlings 138/11/1, 138/7/1 and commercial cultivars like Surajana, Suguna and Sudarsana. Pollen fertility based on stainability was determined in 389/1 and the commercial cultivars. *In vitro* and *in vivo* pollen germination was tested in 389/1. First generation inbreds of 138/11/1(34) and 138/7/1 (5) and two intervarietal hybrid involving 389/1 x Surajana were established.

In vitro pollen germination of 389/1 was tested on B&K medium containing 10% sucrose after standardization. It was observed that among the fertile pollen evidenced by stainability, only 26% actually germinated and produced pollen tubes of considerable length. *In vivo* self pollination indicated germination of pollen on stigmatic surface. Chromosome number analysis was completed in 20 second generation seedling progenies. All of them had 2n=84.

Exploitation of other *Curcuma* species

Though there was no significant variation for strach yield in four *Curcuma* species viz., *Curcuma amada*, *C. aromatica*, *C. xanthorrhiza* and *C. caesia*, the starch granules of the four species differed in size, shape and solubility.

Table 20. Putative candidate genes identified in the curcumin biosynthetic pathway and gene specific primers designed for qPCR

Enzyme code	Name of enzyme (Abbreviation)	Forward primer (3'-5')	Reverse primer (3'-5')
4.3.1.24	Phenylalanine ammonialyase (PAL)	ACA TCC TCG CTT TGC TCG	GTC AAG TGG TCA GTG AAC
1.14.13.11	Cinnamate 4-hydroxylase (C4H)	TTA CTT GCA GGC GGT GAT C	AGG CGT TGA CCA GTA TCT TG
6.2.1.12	4-coumarate: coenzyme A ligase (4CL)	GGA ACA CGA TCG ACA AGG AAG	CCT GAA AAC CCT TGT ACT TGA TG
1.14.14.9	Coumarate 3-hydroxylase (C3H)	CTG GTT TCA CAA ATC GCT TCC	CGA ATC CAT CTT CCG AGT CTG
2.3.1.133	Hydroxycinnamoyl-CoA shikimate/ quinate hydroxycinnamoyl transferase (HCT)	TTC ATC GAC AAC CCC AAG AC	ATC GGA GAC ATT GGG AAG C
2.1.1.104	Caffeoyl CoA O-methyltransferase (CCOMT)	TGA TGT AGT TGT CCT TGT CCG	CAA TTG CTC GAA GAT GCG AAG
2.3.1.211	Diketide CoA synthase (DCS)	CAA CAG CAC GCC CCA GTC GA	GTG CTG TTC ATC CTG GAC GAG

2.3.1.217	Curcumin synthase 1 (CURS1)	TCA GCT CAT CCA TCA CGA AGT ACA C	CATCATTGACGCCATC GAAGC
2.3.1.217	Curcumin synthase 2 (CURS2)	TGT TGC CGA ACT CGG AGA AGA C	TCG GGA TCA AGG ACT GGA ACA AC
2.3.1.217	Curcumin synthase 3 (CURS3)	CCC ATT CCT TGA TCC CTT TTC C	TGG AGC CCT CCT TCG ACG ACC

Curcumin biosynthesis

A rapid method for isolation of good quality total RNA was optimized for constructing normalized full length cDNA library from pooled total RNA from leaf, rhizome, root and pseudostem tissues of turmeric. The presence of isoforms of curcumin synthase I, II and III was confirmed from cDNA from

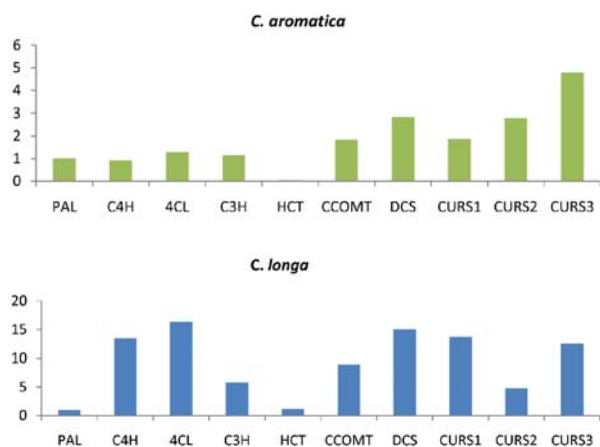


Fig. 30. Expression profile of curcuminoid biosynthetic pathway genes *C. longa* and *C. aromatica*

turmeric rhizomes using gene specific primers. Transcriptome profiling of *C. longa* identified all the key genes of the curcumin biosynthetic pathway (Table 20) and the expression of these genes were confirmed in turmeric tissues by qPCR (Fig. 30). Transcriptome and expression analysis by qRT-PCR revealed the expression of these putative genes involved in curcumin biosynthesis even in *C. aromatica*, devoid of curcumin indicating the role of endogenous factors in regulation of curcumin synthesis. Transcription factors with putative regulatory roles in curcumin biosynthesis like R2R3 MYB, AP2/EREBP and WRKY etc could be

identified. Differential gene expression of *C. longa* vs *C. aromatica* identified three unigenes upregulated above 80 fold in *C. longa* showing similarity to type 2 polyketide synthase of *Musa acuminata*.

miRNA studies

Genome wide identification of miRNAs was done through Illumina deep sequencing. From a single small RNA cDNA library from four month old turmeric rhizomes about 93 conserved and 33 novel miRNAs could be identified. Of these, 10 conserved and 18 novel miRNAs were supported by the presence of miRNA* (opposite miRNA) sequences, which confirms their existence. Two of the *in silico* predicted miRNAs, viz., clo-miR 2 & 4 could be validated by illumina. Stem loop RT and qRT-PCR were used to validate the expression of three miRNAs and their tissue-specific expression patterns. Out of this one miRNA (clo-miR93) was novel and specific to turmeric. miRNA targets were also predicted computationally, many of which were found to be involved in plant growth and development, stress response and metabolism.

SSR markers identification and their cross species amplification

Identified and validated a total of 20 novel polymorphic SSR markers in turmeric from genomic DNA libraries. Cross species amplification could be confirmed in ginger, cardamom and related species of *Curcuma*. Transcriptome analysis identified 5,488 putative SSRs in 99482 contigs. Polymorphic SSR marker CLM33 could distinguish varieties Suguna and Sudarshana from the rest of the released varieties on 15% PAGE. Two SSRs were identified in contigs encoding 4-Coumarate CoA ligase which is involved in curcumin biosynthetic pathway. Transcriptome analysis identified 34497 putative SNPs. After removal of indels and homozygous calls the putative



SNPs were reduced to 17354. A total of 11612 transitions and 5742 transversions were identified. About 202 SNPs in the curcumin biosynthetic pathway genes from *C. longa* were identified. Besides, 100 accessions of turmeric were field planted and morphological characters including rhizome characteristics were recorded for the second year.

Organic farming

Turmeric was grown by applying standardized organic package in comparison with the integrated and chemical systems to test their sustainability. The activity of acid, alkaline and phosphodiesterase enzymes were higher under organic system on par with that of integrated system. Dehydrogenase was significantly highest under organic system. Soil available P, K and Cu contents were higher under inorganic and integrated systems and that of OC, Ca and Mg were higher under organic system. The rhizome yield was higher under inorganic followed by integrated systems (10 and 9.1 Kg 3 m², respectively).

Adulteration detection using DNA bar coding

Five accessions each of *C. longa* (Accs. 143, 119, 126, 360, 361), *C. xanthorrhiza* (Accs. 1123, 1163, 1164, 1167, 1168), four accessions of *C. zedoaria* (Accs. 465, 760, 765, 1517), four samples of cassava

Table 21. SNPs that discriminates *C. longa* from *C. zedoaria*

Species	Position of SNP and nucleotides substituted			
	293	388	410	439
<i>C. longa</i>	G	G	G	G
<i>C. zedoaria</i>	A	A	T	C

starch and five market samples of turmeric were taken for the study. High quality amplifiable DNA was isolated from the above samples and the PCR conditions are optimized for the barcoding loci, *rbcL* and *ITS*. The samples showed 100% PCR and sequencing success for both the loci. Sequencing analysis of *ITS* and *rbcL* revealed the presence of single nucleotide polymorphisms (SNPs) specific to cassava starch in one of the turmeric market samples thereby confirming the adulteration of market samples with cassava starch. Blast analysis also revealed the close similarity of the market sample with cassava starch. It was found to be ideal for adulterant detection as the number of polymorphic sites were high in this locus. Sequence analysis of *ITS* locus showed the presence of four SNPs at positions 293, 388, 410 and 439 specific to the adulterant *C. zedoaria*, which clearly discriminated it from *C. longa* (Table 21). However, *rbcL* locus could not distinguish between these two species. The generated barcodes are deposited in GenBank database.



VANILLA

Genetic resources

Ninety three germplasm collections and 400 seedling progenies /interspecific hybrids are being maintained in conservation nursery.

Interspecific hybrids

Interspecific hybrids involving *V. planifolia* x *Vanilla* sp. (Andamans) and *V. planifolia* x *V. tahitensis* and 15 plants of *V. planifolia* x *V. aphylla* were inoculated with *Fusarium oxysporum*. Three lines that survived after three rounds of inoculation are being maintained for third round of inoculation.

Charaterisation

Morphological characters namely leaf length, leaf breadth and internode length were recorded from 10 interspecific hybrids involving *V. planifolia* and *V. tahitensis* and 10 interspecific hybrids involving *V. planifolia* and *V. aphylla*. Twelve interspecific hybrids involving *Vanilla* sp. (Andamans) and *V. aphylla* were characterized based on morphological characters (Table 22 & 23). Chromosome number analysis was completed in twelve interspecific hybrids between *Vanilla* sp (A&N) x *V. aphylla* and five interspecific hybrids between *V. planifolia* and *V. aphylla*. In interspecific hybrids between *Vanilla* sp (A&N) x *V. aphylla* chromosome number was $2n=56$ while in parents it was $2n=40$ and $2n=72$ respectively. However, the interspecific hybrid between *V. planifolia* and *V. aphylla* showed $2n=28$ most frequently, the chromosome number of female parent (*V. planifolia*).

Twelve interspecific hybrids between a wild *Vanilla* sp. and a leafless species *Vanilla aphylla* were characterized after flowering, based on plant morphology, flower characters and chromosome number. All the 12 hybrids had intermediate leaf size compared to the parents and varied in measurements between them. All the interspecific hybrids showed more than 95% pollen sterility compared to both the parents which had about 50 % pollen fertility. Self

pollination of the hybrids resulted in total absence of fruit set while back cross with both the parents resulted in 100 % fruit set indicating the expression of sterility is at the level of pollen grains alone. The interspecific hybrids also produced successful fruit set on pollination with a pink flowered variant of *Vanilla* sp. (A & N Islands) and also other wild species namely *Vanilla ptilifera*. Chromosome number analysis

Table 22. Morphological characters of interspecific hybrids between *Vanilla* sp. (A&N) and *V. aphylla*

Genotype	Leaf length (cm)	Leaf breadth (cm)	Internode length (cm)	Stem girth (cm)
<i>Vanilla</i> sp (A&N) (Female parent)	17.79	5.0	9.92	3.36
<i>V. aphylla</i> (Male parent)	No Leaf	No Leaf	12.70	2.81
Hybrid 1	12.05 ab*	1.93 b	10.95 a	2.82 abc
Hybrid 2	10.75 cd	1.61 cd	10.65 a	2.95 a
Hybrid 3	12.66 a	1.96 b	10.85 a	2.73 abcd
Hybrid 4	11.51 bc	1.98 b	10.68 a	2.79 abc
Hybrid 5	07.58 f	1.13 e	10.22 a	2.64 bcd
Hybrid 6	10.46 d	1.62 cd	10.88 a	2.54 cd
Hybrid 7	10.73 cd	1.71 c	10.80 a	2.82 abc
Hybrid 8	11.78 ab	1.68 c	10.59 a	2.83 ab
Hybrid 9	09.52 e	1.46 d	10.19 a	2.79 abc
Hybrid 10	10.23 de	1.51 d	09.86 a	2.48 d
Hybrid 11	11.55 bc	1.58 cd	10.59 a	2.47 d
Hybrid 12	12.44 ab	2.20 a	10.79 a	2.87 ab

*Means followed by the same letter are not significantly different in DMRT at $\alpha=0.05$

revealed that the interspecific hybrids had $2n=56$ as the most frequently occurring chromosome number while the chromosome number of *Vanilla* sp (A & N Islands) was $2n=40$ and that of *V. aphylla* was $2n=72$.

Maintenance of *in vitro* cultures of vanilla and *ex vitro* establishment

In vitro cultures of 100 seedlings from seven collections were maintained. Twenty interspecific hybrids between *V. planifolia* and *V. aphylla* and 160 seedlings from selfed seeds of five accessions were established *ex vitro*.

Table 23. Comparison of floral appendages of interspecific hybrids of *Vanilla* sp. (Andamans) x *V. aphylla* and parents

Genotype	Sepal 1 (mm)	Sepal 2 (mm)	Sepal 3 (mm)	Petal 1 (mm)	Petal 2 (mm)	Labellum length (mm)	Labellum width (mm)	Length of column (mm)	Length of ovary (mm)	Anther size (mm)
<i>Vanilla</i> sp (A&N)	48.33 ^{a*}	47.33 ^a	47.33 ^a	48.00 ^a	48.00 ^a	43.33 ^a	23.67 ^a	36.33 ^a	29.67 ^e	2.5 x 3
<i>V. aphylla</i>	27.67 ^h	26.67 ^f	26.67 ^g	27.67 ^e	27.33 ^f	26.00 ^g	16.00 ^g	17.00 ^e	29.33 ^e	1.5 x 2.5
Hybrid-1	33.67 ^{efg}	32.33 ^{de}	32.33 ^{def}	34.33 ^{cd}	34.33 ^{de}	31.33 ^{cdef}	19.33 ^f	23.33 ^{bed}	35.67 ^{cd}	2 x 3
Hybrid-2	39.67 ^b	37.33 ^b	37.33 ^b	40.67 ^b	40.67 ^b	37.00 ^b	22.33 ^{ab}	26.33 ^b	34.33 ^{cd}	2 x 3
Hybrid-3	33.00 ^{fg}	32.00 ^{de}	32.00 ^{ef}	34.00 ^d	34.00 ^{de}	30.00 ^f	19.67 ^{ef}	24.33 ^{bc}	36.00 ^{bc}	2 x 3
Hybrid-4	32.33 ^g	31.00 ^e	31.00 ^f	33.33 ^d	33.33 ^e	30.33 ^{ef}	19.33 ^f	23.67 ^{bed}	36.33 ^{bc}	2 x 3
Hybrid-5	36.00 ^{cde}	33.33 ^{cde}	33.33 ^{cdef}	35.33 ^{cd}	35.33 ^{cde}	33.33 ^{cdef}	20.00 ^{def}	24.33 ^{bc}	31.00 ^{de}	2 x 3
Hybrid-6	33.00 ^{fg}	31.00 ^e	31.00 ^f	33.67 ^d	33.67 ^{de}	30.67 ^{def}	20.33 ^{cdef}	24.00 ^{bc}	33.33 ^{cd}	2 x 3
Hybrid-7	37.00 ^{cd}	35.33 ^{bc}	35.67 ^{bc}	36.00 ^{cd}	36.00 ^{cd}	34.67 ^{bc}	21.67 ^{bed}	20.67 ^d	35.33 ^{cd}	2 x 3
Hybrid-8	36.33 ^{cd}	34.67 ^{bed}	34.67 ^{bede}	36.00 ^{cd}	35.67 ^{cde}	34.33 ^{bc}	21.33 ^{bcd}	22.00 ^{cd}	33.33 ^{cde}	2 x 3
Hybrid-9	34.67 ^{defg}	33.67 ^{cde}	33.67 ^{cdef}	35.33 ^{cd}	35.33 ^{cde}	32.67 ^{cdef}	20.00 ^{def}	23.67 ^{bed}	41.33 ^a	2 x 3
Hybrid-10	35.33 ^{cdef}	33.33 ^{cde}	33.00 ^{cdef}	35.00 ^{cd}	35.00 ^{cde}	33.33 ^{cdef}	21.00 ^{bedef}	23.33 ^{bed}	40.33 ^{ab}	2 x 3
Hybrid-11	37.00 ^{cd}	35.33 ^{bc}	35.33 ^{bc}	36.00 ^{cd}	36.00 ^{cd}	34.00 ^{bed}	22.00 ^{abc}	21.33 ^{cd}	40.33 ^{ab}	2 x 3
Hybrid-12	37.33 ^c	35.33 ^{bc}	35.00 ^{bed}	37.00 ^c	37.00 ^c	33.67 ^{bede}	21.00 ^{bedef}	22.67 ^{cd}	34.33 ^{cd}	2 x 3

*Means followed by the same letter are not significantly different in DMRT at $\alpha=0.05$



TREE SPICES - NUTMEG

Genetic resources

Surveys were conducted in Kottayam, Kozhikode and Malapuram districts of Kerala, Uttarkannada District of Karnataka, Ratnagiri District of Maharsathra and 35 nutmeg accessions, two in cinnamon and two in garcinia were collected. The collection included 14 monoecious nutmegs, a seedless nutmeg, yellow mace nutmeg, white kokum, released varieties of nutmeg and cinnamon, high yielding nutmeg etc.

Release of new variety

A new nutmeg variety IISR- Keralashree was approved for release at the 24th AICRPS workshop held on 24-26 October 2013, at Jagudan, Gujarat. This is the first nutmeg variety developed by farmer participatory breeding programme (Table 24 and Fig. 31).



Fig.31. Seed with mace (IISR Keralashree (left) and IISR Viswashree (right)); and Nut (IISR Keralashree (Left) and IISR Viswashree (right))

Table 24. Morphological and yield characters of IISR Kerala sree

Morphological characters	
Plant height of graft	4.5 to 5.0 m at 10 years
Stem girth at 60 cm	25.00 cm
Leaf size	Medium
Leaf shape	Elliptic
Age at first flowering of graft	4 years after planting
Flowering	Profuse
Percentage of male flowers	0
Percentage of female flowers	100
Arrangement of flowers	Single, rarely, seen in clusters of 2
Colour of ripe fruit	Yellow
Colour of aril	Dark red
Colour of seed	Brownish black
Shape of fruit	Elongate/oblong
Size of nut	Bold
Mace	Entire, thick and dark red

Yield and quality characters

Fresh weight of fruit	75-100 g
Fresh weight of seed	13-16 g
Dry recovery of nut	70%
Fresh weight of aril	4.5 to 6.0 g
Dry recovery of aril (mace)	35%
Mean yield graft ⁻¹ at 10 th year after planting	2000 fruits
Dry nut yield graft ⁻¹ at 10 th year after planting	21 kg
Dry nut yield ha ⁻¹ @ 360 graft	7560 kg
Maceyield graft ⁻¹ at 10 th year after planting	4.2 kg
Mace yield ha ⁻¹ @360 grafts	1512 kg
Essential oil in nut (%)	5.9
Essential oil in mace (%)	7.5
Oleoresin in nut (%)	9.1
Myristicin in nut oil (%)	1.6
Myristicin in mace oil (%)	9.4
Elemicin in nut oil (%)	1.4
Elemicin in mace oil (%)	0.07
Total fat content (%)	24.9
α- pinene in nut oil (%)	7.1
α -pinene in mace oil (%)	4.7
Sabinene in nut oil (%)	35.4
Sabinene in mace oil (%)	29.4

Comparative evaluation of yellow mace type nutmeg with red coloured type

Various growth parameters of the nutmeg variant with yellow mace were evaluated. The fresh weight of fruit ranged from 70-100g; fresh mace weight from 3-5g; fresh nut weight from 7.5 -11.5g; dry recovery of nut was 68%; dry recovery of mace was 30%; colour of dried mace was yellow and 100% germination was observed when seeds were sown. The biochemical quality was similar in both the types.

Biochemical characterization

Essential constituents of oil of red and yellow mace type accessions indicated sabinene, pinenes, limonene, α-terpineol and myristicin as chief constituents. The essential oil from red-coloured mace contained 24-28% sabinene, 9-10 % α- pinene, 6-7% limonene, 12-26% myristicin and 0.5-2.0 % elemicin and 3-5 % safrole besides other minor constituents. Some distinct accessions IC 548921 (21.5% myristicin and 10.7% elemicin), IC 548916 (13.2% myristicin and 17.7% elemicin), IC 548918

(13.2% myristicin and 14.2% safrole) and IC 645944 (18.2% safrole and 11.0% elemicin) were identified to have high elemicin and safrole. Seedless red mace type collected from Kottayam showed 11.7% α -pinene, 16.9% sabinene, 6.6% β -pinene, 3.3% myrcene, 6.7% limonene, 4.0% 4-terpineol, 2.1% safrole, 26.0% myristicin and 2.0% elemicin.

The essential oil from yellow-coloured mace contained 27-30% sabinene, 8-10% α -pinene, 6-7% limonene, 2-3% myristicin, 14-16% elemicin and 0.9-1.0% safrole. IC-645944, yellow coloured mace from Appangala also contained low myristicin (3.6%) and high elemicin and safrole. The essential oil from nut had similar composition as that of mace. The oil from nuts with yellow coloured mace nutmegs also showed high elemicin (16.5%) and low myristicin (7.6%). The essential oil of pericarp of both red and yellow mace was low in sabinene and high in α -terpineol and 4-terpineol content compared to that of nut and mace. However, the essential oil of pericarp with yellow mace contained higher level of elemicin compared to that of red mace (5.1%). The antioxidant activity (DPPH and phosphomolybdenum methods) of both nut and mace oils showed positive correlation with myristicin level.

Hormone assisted ripening of nutmeg

Experiments were conducted to study the influence of growth regulators ethylene and NAA on fruit

splitting in nutmeg. The study revealed that dipping fruits in ethrel (at 500 and 1000 ppm for 10 minutes) was very effective in splitting of matured nutmeg fruits and 100% splitting could be achieved in 24h after treatment. NAA (at 25 and 50 ppm) was also effective which induced about 80% fruit splitting in 24h after treatment. Control (water dipping) induced about 50% fruit splitting. It took 8-10 days to attain around 80% splitting in control and water dip treatments.

Adulteration detection using DNA bar coding

Amplifiable DNA was isolated from genuine and market samples of nutmeg mace. PCR conditions for *rbcL* locus was standardized (Fig. 32).

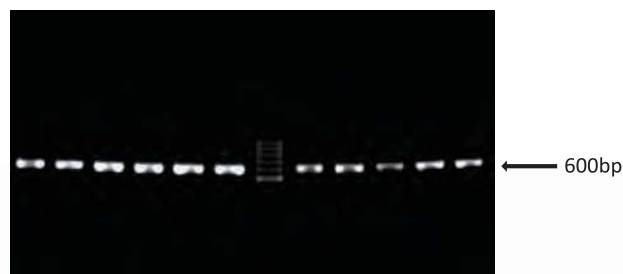


Fig. 32. PCR amplification of *rbcL* locus in nutmeg. Lane 1 to 3 - *M. fragrans*; lane 4 to 6 - *M. malabarica*; lane 7 - 100 bp ladder; lane 8 - market sample 1; lane 9 - market sample 2; lane 10 - market sample 3; lane 11 - market sample 4; lane 12 - market sample 5.



CINNAMON

Adulteration detection using DNA bar coding

Bark DNA was isolated from *C. verum* (IC.No. 37151, 370125, 370177, 370179, 370167), *C. cassia* (*C. aromaticum*) (IC No. 370417, 370412, 370429, 370401, 370408) and *C. malabatum* using a new protocol developed (Fig. 25).

PCR parameters for the barcoding loci viz., *rbcL*, *matK* and *psbA-trnH* were standardized. The PCR

Table 25. The average intraspecific and interspecific distances of *C. verum* and their adulterants

Distance		Locus	
Intraspecific distance	Species	<i>rbcL</i>	<i>psbA-trnH</i>
	<i>C. verum</i>	0	0.269
Interspecific distance	<i>C. verum</i> & <i>C. cassia</i>	0.198	0.352
	<i>C. verum</i> & <i>C. malabatum</i>	0.007	0.194

success rate, sequencing efficiency, inter and intra specific divergence and occurrence of single nucleotide polymorphisms (SNPs) were utilized to assess the potential of each barcode loci to authenticate *C. verum* from its related adulterants.

The amplification and sequencing success was 100% for *rbcL* and *psbA-trnH* while *matK* failed to amplify the market samples. MEGA analysis of *rbcL* locus showed higher interspecific divergence while *psbA-trnH* exhibited lower interspecific divergence (Table 25). The presence of the adulterant *C. cassia* (*C. aromaticum*) could be detected in two out of 10 market samples studied.

Ten barcodes of *rbcL* and *psbA-trnH* loci from *C. verum* and *C. cassia* were deposited in NCBI database.

SNPs specific to *C. cassia* (*C. aromaticum*) were detected in *rbcL* locus in two out of the ten market samples studied thereby confirming the presence of *C. cassia* adulteration in commercial samples of true cinnamon. Out of the three loci, *rbcL* locus proved to be efficient in tracing out adulterants in traded cinnamon. The SNP sites in this locus can be exploited in designing *C. cassia* specific primers, enabling kit development for easy detection of adulterants at the band level itself thereby bypassing the cost of sequencing.

C. malabatum adulteration was not detected in any of the traded samples under study. Ten barcodes of *rbcL* and *psbA-trnH* loci from *C. verum* and *C. aromaticum* were deposited in NCBI database.





GARCINIA

Nutraceutical properties of fruit of selected Indian *Garcinia* spp.

The dried fruit were finely powdered and digested using di-acid reagent (HNO₃ and HClO₄ in the ratio 9:4). It was found that Mg and K are the major minerals present in *Garcinia* (Table 26).

Vitamins and flavanoids in *Garcinia* fruit rind

Fat soluble vitamins were extracted from fruit using methanol-chloroform (1:1) and water soluble vitamins were extracted by using phosphate buffer of pH 7.5. The amount of vitamins present in the extracts was determined in a UV-Vis spectrophotometer using their respective molar extinction coefficient values. The total vitamin content was highest in *G. mangostana* (61 mg 100g⁻¹), followed by *G. pedunculata* (36 mg 100g⁻¹). Other than ascorbic acid,

the vitamin content showed only a small variation (<10%) among the species studied (Table 27).

The content of flavonoids varied from 0.9-3.7 g 100g⁻¹ and xanthenes from 0.91 -2.66g 100g⁻¹. *G. indica* had the highest content of flavonoids but lowest xanthone content, while *G. xanthochymus* had highest xanthenes content.

GIS study

Based on the collections made in Western Ghats and Himalayan foot hills, the domain of *Garcinia* in India was predicted with the help of Ecocrop model of DIVA GIS. The prediction indicated that other than the surveyed area, Andaman and Nicobar islands, parts of Odisha, Uttaranchal and Himachal Pradesh are the suitable domains for *Garcinia* (Fig. 33).

Table 26. Mineral compositions of *Garcinia* fruits

Sample	Na (mg 100g ⁻¹)	K (mg 100g ⁻¹)	Ca (mg 100g ⁻¹)	Mg (mg 100g ⁻¹)	Fe (mg 100g ⁻¹)	P (mg kg ⁻¹)
<i>G. gummi-gutta</i>	2.88	26.6	12.67	14.35	9.00	5.34
<i>G. indica</i>	1.55	44.5	13.21	33.45	12.06	4.51
<i>G. mangostana</i>	2.58	78.3	5.82	60.43	9.02	7.45
<i>G. xanthochymus</i>	2.06	28.4	13.07	30.62	10.82	3.48
<i>G. subelliptica</i>	1.52	43.3	12.33	34.45	9.00	5.43
<i>G. kydia</i>	2.54	38.7	12.54	25.25	10.00	4.32
<i>G. lanceaefolia</i>	1.35	52.3	12.54	30.23	9.00	3.64
<i>G. pedunculata</i>	2.48	27.3	13.21	35.43	10.12	4.32

Table 27. Vitamin composition of *Garcinia* fruits

Sample	Thiamine (B1) (µg 100g ⁻¹)	Riboflavin (B2) (µg 100g ⁻¹)	Niacin (B3) (µg 100g ⁻¹)	Ascorbic acid (C) (mg 100g ⁻¹)	Vitamin B12 (µg 100g ⁻¹)
<i>G. gummi-gutta</i>	48	275	45	14.35	8.75
<i>G. indica</i>	52	320	63	33.45	12.06
<i>G. mangostana</i>	50	300	60	60.43	9.52
<i>G. xanthochymus</i>	37	250	50	30.62	10.76
<i>G. subelliptica</i>	50	281	45	34.45	9.03
<i>G. kydia</i>	47	267	50	25.25	10.15
<i>G. lanceaefolia</i>	52	283	45	30.23	8.02
<i>G. pedunculata</i>	49	276	47	35.43	8.12

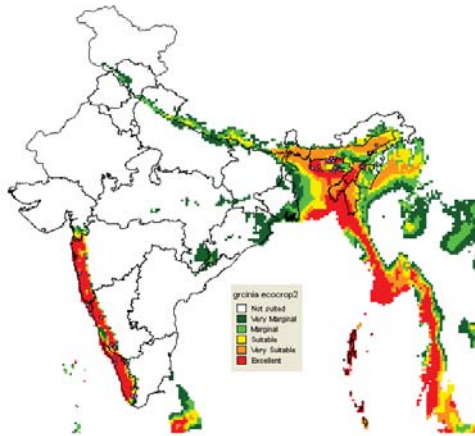


Fig. 33. Garcinia ECOCROP MODEL showing the Adobe for Garcinia.

Production of food extrudates

Rice flour and five different spice powders (ajwain, black pepper, white pepper, turmeric and dry ginger), each blended in the ratio of 96:4 at a moisture content of 13.9% were conditioned at 4^oC for three days and extruded. The extrusion process was carried out in a lab model twin screw extruder at a constant die temperature of 140^oC and a screw speed of 350 rpm. Comparison of rice flour alone and extrudates from flour spice blends were done based on their functional, biochemical, physical, textural and sensory properties. Rice flour blended with ajwain and dry ginger gave better preferred extrudates based on their overall acceptability scores of 6.7 and 6.6, respectively. Rice flour blended with dry ginger extrudates showed the lowest water absorption index of 4.21 and higher hardness of 13.91N.



SOIL FERTILITY STATUS OF SPICE GROWING SOILS

Soil based nutrient management plans for Kozhikode district

Around 18000 soil samples from 74 Panchayats were analyzed for physico-chemical properties and uploaded into www.keralasoilfertility.net for generating nutrient advisory cards for individual farmers. The salient findings are as under:

Frequency of soil acidity (pH) classes

About 49% of the samples analyzed were found to be strongly acidic, of which 4% samples were extremely acidic (Fig. 34). Nearly 39% and 10% of the soils were found to be moderately and slightly acidic to neutral, respectively. In general, all the soils are in need of immediate application of amendments like lime/ dolomite to correct the pH, which in turn would facilitate balanced nutrient availability.



Fig. 34. Frequency of soil acidity classes across Kozhikode District, Kerala

Frequency of available major nutrient classes

In majority of the samples (54%) OC was in the medium levels followed by low (30%) and adequate (16%). In line with the OC content we may assume that the available N content will also follow a similar pattern, where 54% of soils analyzed will be of medium category. With regard to P, 21% and 18% of the soils showed low and medium availability, respectively (Fig. 35). The remaining 61% of the soils were of high category, of which 25% and 27% of the soils have very high and extremely high P status. These high levels of P may cause imbalance in the availability of other nutrients, especially that of Zn. Fifty percent of the analyzed samples were found to be medium in available K (<220 kg ha⁻¹) and 26% and 24% of the soils showed high and low status,

respectively. Nine percent of the soils registered very low available K status (<60 kg ha⁻¹), which needs immediate attention.

Frequency of available secondary and micro nutrient classes

The S status was high or adequate in 51% of the soils followed by medium (23%) and low (20%). The Ca status was found to be low in 48% of soil samples analyzed, having 9% of soils under very low category with <500 ppm available Ca, which needs immediate ameliorative measures (Fig. 36).

The available Mg status was alarmingly low with 81% of the samples falling in low category, of which 42% of soils were found to be very low. Only 19% of soils were in adequate category. The soil available Zn was found to be adequate to support the crop requirement in 92% of samples with only 7% of samples falling under low category. Similarly, 97% of soils were also found to be high or adequate in its Cu availability. With regard to B, 77% of soils in the district were found to have sufficient levels and 19% were deficient with < 1.0 ppm.

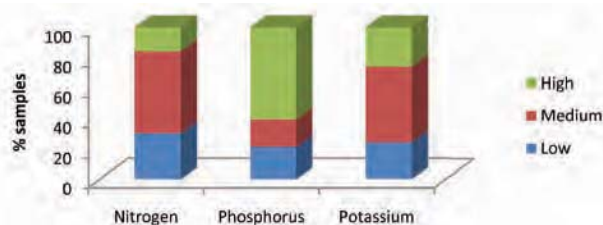


Fig. 35. Frequency of available major nutrient classes across Kozhikode District, Kerala

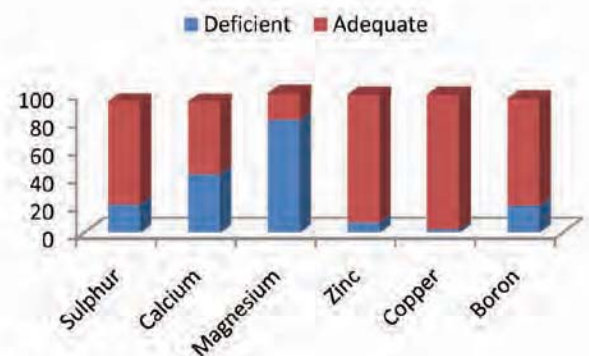


Fig. 36. Frequency of available secondary and micro nutrient classes across Kozhikode District, Kerala

EXTENSION AND IMPACT ASSESSMENT

Technology mission for pepper in Wayanad

Twenty farmer's plots were selected in Mepadi and Vythiri panchayats of Wayanad district and FLDs on technologies to rejuvenate yellowing of black pepper was initiated. The inputs like planting material, lime, organic manures, neem cake, micro nutrient mixtures and bio agents were supplied to both the old and new FLD plots and nine visits were made by team of scientists to hot spots/ problem areas along with KAU scientists and advisories were given to the farmers. The yellowing status of the crops have improved from Moderate - high (3-4 scores) to Nil-mild (0-1 score) category in all the four panchayats studied (Fig. 37). The spike intensity (measured in 50 cm²) and bulk density of the dry pepper significantly improved in treated vines as compared to untreated vines in all the panchayats. Ten farmer's nurseries were given at Vythiri and Mepadi panchayats to promote participatory mode of planting material production.

Integrated black pepper development in North Kerala districts

Soil samples and basic information were collected from 40 plots from four Panchayats viz., Koorachundu, Chakittupara, Thamarasery and Olavanna of Kozhikode district where FLDs are to be established on production technologies and varieties of black pepper. Five farmers from each panchayat were identified for establishing black pepper nurseries and nucleus planting materials and training for maintaining the nurseries were given to them.

Varietal spread in turmeric and ginger

Feedbacks from farmers' plot revealed very high yield of two released turmeric varieties. A farmer (Mr. Sivenesan, Gundlupet, Karnataka) recorded an yield of about 40.0 t acre⁻¹ for IISR Alleppey Supreme (Fig. 38) while the highest yield reported in case of IISR Prathibha is 27.0 t acre⁻¹ (Mr. Sashikant Jadhao, Chandrapur, Maharashtra) (average yield of these varieties at the Research farm is about 35-40 t ha⁻¹). A front line demonstration of IISR Prathiba variety of turmeric was conducted in four farmers filed in Guntur district under the National Horticultural Mission.

Farmers adopted scientific cultural practices like organic soil amelioration, balanced application of chemical fertilizers, irrigation and plant protection measures. An average yield of 40 t ha⁻¹ was recorded in the demonstration plots. IISR Prathibha was showing



Fig. 37. FLD on management technology of black pepper at farmer's field in Wayanad

vigorous vegetative growth and high input responsiveness that contributed to stable and higher yield compared to local varieties like Kadappa and Tekurpeta. To synergise the adoption process of improved varieties of turmeric and make farmers updated on scientific cultivation a two day training programme was organized at Vijayawada in January 21-22, 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.

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



Fig. 38. Mr. Sivenesan (extreme right) in his IISR Allepey Supreme plot



Fig. 39. IISR Prathiba plot of Mr. Sashikant Jadhao, Chandrapur, Maharashtra



ALL INDIA COORDINATED RESEARCH PROJECT ON SPICES

The All India Coordinated Research Project on Spices (AICRPS) located in Kerala with its head quarters at IISR, Kozhikode is giving emphasis on 12 major spices at 19 regular, 8 co-opting and 7 voluntary centres spread over 21 states of the country. The annual budget as ICAR share for the year, 2013-14 was Rs. 396 lakhs.

Genetic resources

Fifty new collections were made from black pepper, cardamom, ginger, turmeric and nutmeg genotypes were made during the year. Evaluation of black pepper germplasm at Panniyur indicated the cultivar PRS 64 ranked first with 3.11 kg green berry yield. Among the 36 accessions of black pepper, the accession PN 56 (46.5 m²) and PN 70 (44.5 m²) were found to be promising with respect to number of fruiting spikes per square meter at Yercaud. Among top 10 high yielding accessions of cardamom at Pampadumpara, HY 10 registered maximum dry yield followed by MCC 18 and HY 13. Among the 24 accessions of nutmeg maintained at Pechiparai, the accession MF- 4 recorded maximum number of fruits and dry mace yield.

Crop improvement

Improved turmeric variety Duggirala Red (JTS 6) from Dr. YSRHU, Kammarpally centre; nutmeg variety (Keralashree), the first spices variety developed in farmers participatory breeding mode from IISR, Kozhikode; LCC 234 off season coriander variety suitable for protected cultivation from Dr. YSRHU, Guntur; HM 348, a fenugreek variety from CCSHAU, Hisar were recommended to release during the XXIV workshop held at CRSS, Jagudan.

The hybrids PRS 160 and PRS 161 were found to be promising with maximum green berry yield of 3.5 kg vine⁻¹ and 3.1 kg vine⁻¹ respectively, in Panniyur. In the CVT, HB 20052 recorded the maximum green berry yield of 2.9 kg vine⁻¹ that was on par with ACC.57 and C 1090 (2.2 kg vine⁻¹). In the grafting trial at Panniyur maximum green berry yield (1.63 kg) was recorded by runners of Panniyur 1 grafted on *Piper colubrinum*.

Wide variation in yield was noticed in CVT trial on cardamom at various centres. The clone IC 349545 recorded maximum yield (731 kg ha⁻¹) at Appangala, IC 349587 (816.2 kg ha⁻¹) at Sakaleshpur, IC 346951 (310 kg ha⁻¹) at Mudigere and PL 14 (583.3 kg ha⁻¹) at Myladumpara.

In an experiment genotype x environmental interaction in ginger, maximum yield was recorded by Himgiri (16.7 t ha⁻¹) at Mizoram, Surabhi (32 t ha⁻¹) at Pasighat, S 646 (26.9 t ha⁻¹) at Pottangi and SG-26-04 (21 t ha⁻¹) at Solan.

Out of seven turmeric genotypes tested in a CVT turmeric trial at Kammarpalli, NDH 790 recorded maximum fresh rhizome yield (27.72 t ha⁻¹) whereas the entry Acc 48 (17.28) recorded maximum yield at Raigarh and found moderately resistant to leaf blotch and leaf spot diseases.

In cassia evaluation at Pechiparai, among the four selections, D3 was found to be promising with maximum plant height (5.90 m), stem girth (40.79 cm), leaf yield (396.78 g tree⁻¹) and bark yield (226.12 g tree⁻¹).

In coriander CVT 2012 at Guntur, COR 46 (1300 kg ha⁻¹) recorded maximum yield.

Crop production

Experiments on organic cultivation of spices based cropping system at Panniyur has shown that the recommended package of FYM 10kg, NPK 50:50:200 g plant⁻¹, recorded significantly higher green berry yield (2.72 kg vine⁻¹) compared to organic package (1.68 kg vine⁻¹).

In a drip fertigation experiment at Panniyur (50% RDF + 8 L drip) resulted in higher spike yield (7.75 kg/vine) and green berry yield (5.43 kg vine⁻¹) in black pepper that was on par with 75% RDF + 8 L drip. Application of irrigation to small cardamom 9 L clump⁻¹ day⁻¹ along with 100% recommended dose of fertilizer through drip recorded the highest capsule yield (220.35 kg ha⁻¹) at Mudigere that was on par with irrigation 9 L clump⁻¹ day⁻¹ with 75% recommended fertilizer dose (215.25 kg ha⁻¹).



In an another nutrient trial on cardamom at Mudigere application of FYM (30 t ha⁻¹) + recommended NPK (125 :125 : 250 kg ha⁻¹) recorded maximum dry capsule yield (255.66 kg ha⁻¹).

In a trial standardization of water requirement of turmeric through drip irrigation, drip once in a day at 80% pan evaporation (PE) recorded maximum rhizome yield at Kammarpalli and Guntur (74.7 t ha⁻¹ and 49.9 t ha⁻¹ respectively).

Among the PGPR bioformulations evaluated for coriander, seed treatment with combination of FK14 (*Pseudomonas putida*) and FL18 (*Macrobacterium paraoxydans*) recorded maximum yield in coriander (1191.9 kg ha⁻¹) at Guntur whereas maximum seed yield of 8.7 and 9.20 q ha⁻¹ was found at Raigarh when seeds were treated with Rhizobacteria FK14 and FL18 respectively. Maximum yield of fenugreek was recorded when seeds treated with combination of FK14 (*Pseudomonas putida*) and FL18 (*Macrobacterium paraoxydans*) (1233.8 kg ha⁻¹). In fennel at Raigarh maximum seed yield of 8.5 and 9.5 q ha⁻¹ was recorded in rhizobacteria FK14 and FL18 respectively.

Crop protection

In a trial management of *Phytophthora* foot rot of black pepper in existing plantation at Mudigere, spraying and drenching of 0.2 % Kocide 10 days after application of *T. harzianum* @ 50 g + 1 kg neem cake vine⁻¹ was found more effective in reducing the disease incidence with higher yield of 472.5 g vine⁻¹ which was on par with spraying and drenching of 0.1% Sectin + *T. harzianum* (50 g) + Neem cake 1 kg vine⁻¹.

At Pampadumpara lowest disease incidence of pseudostem rot of cardamom was observed by the application of bavistin (2 g L⁻¹) as foliar spray as well as basal application. In an another trial on ginger at Pampadumpara, minimum soft rot incidence was

observed when rhizomes were treated with IISR GRB followed by soil fumigation with cabbage whereas maximum yield was noticed in soil fumigation using cabbage. In an another trial minimum bacterial wilt incidence was noticed in rhizomes treatment by heat followed by soil treatment with bleaching powder whereas maximum yield was recorded by the treatment soil fumigation using cabbage.

This year two new centers one at Mandor, Rajasthan, and another at Sanand, Gujarat were initiated for seed spices in voluntary mode as per XXIV workshop to evaluated seed spices germplasm.

All the 16 released varieties of black pepper were introduced to *Kolli Hills* of Tamil Nadu and Western dry region of Orissa under the supervision of TNAU, Coimbatore and OUAT, Pottangi respectively, to increase the crop diversity in black pepper plantation.

About 24 released varieties of turmeric were distributed to five AICRPS centers for conservation, multiplication and distributions.

Ten Nepal collection of ginger with low fiber were collected from NAGS at IISR, Kozhikode and distributed to five centers in NE for further multiplication and evaluation.

Graft of one high yielding farmer's variety of nutmeg, one seed less nutmeg, one bisexual nutmeg and one nutmeg with yellow mace were collected and distributed to AICRPS centers at Kerala, Tamil Nadu and Maharastra to establish speciality mother garden.

Draft GAPS were finalized is small cardamom for reducing the pesticide residues.

Multiplied gall wasp resistant *Erythria subumbrans* identified by Mudigere center on black pepper standard and distributed to IISR, IIHR and other pepper growing centers in Kerala and Karnataka.

BIOINFORMATICS

Developing and updating databases

Phytophthora-Piper Transcriptom DB: Transcriptome data of *p. nigrum* plants challenged with *Phytophthora capsici* has been developed. This database provides access to the transcriptome sequences, number of genes, functional annotation, gene ontology annotations of *P.nigrum* plants challenged with *P. capsici*.

Ginger Transcriptome Database: Database contains information about transcriptome sequencing and other details such as SNPs, SSRs of *Zingiber officinale* and *Curcuma amada* rhizome tissue samples after challenge inoculation with *R. solanacearum*.

Sequence Repository of IISR: Sequence Repository of IISR is a database designed to store the sequence information from the projects carried out at Indian Institute of Spices Research. Information like sequences and its related information will be stored and displayed (Fig. 40).

Phytophthora whole genome sequence data assembly and annotation

De novo hybrid assemblies using sequence reads from two NGS platforms (Illumina and Roche/454) were made for both isolates of *Phytophthora*. The *de novo* hybrid assembly of two next-generation sequencing (NGS) technologies (Illumina and Roche/454 sequencing) yielded 63.8 Mb genome size at an N50 contig length of 4724 kb, with contig lengths ranging from 200- 42,775. The *de novo* hybrid assembly gives out 32,044 contigs and 4,72,80,344 bases using Newbler Assembler. A reference assembly was also conducted to compare *P. capsici* genome of Joint Genome Institute and percentage identity was 95.35% with an average read depth of 50x. Variants were detected using probabilistic variant detection algorithm in CLC Workbench after removing duplicates. There were 25,569 variants compared the reference, of which 4,113 were deletions, 8621 were insertions and 11,826 were SNPs. On filtering the variants using filter marginal variant calls a final 6316 variants were obtained which consisted of 2134

insertions, 1152 deletions and 2743 SNPs. Structural annotation was carried out using *ab-initio* gene prediction methods and an approximate of 22,358 coding sequences and 54,485 exons were obtained. Simple sequence repeats (SSR) analysis revealed that there are 1344 SSRs out of 32,044 contig sequence analysed. Whole genome alignment and comparison with reference genome revealed 1,298,146 SNP sites. There were 917 genes are common with reference genome of *P. capsici* (JGI), and 5501 genes are unique in *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.

Genome mining of endophytic bacteria

Based on QSAR screening, the secondary metabolites of *Bacillus megaterium* and *Pseudomonas putida* were grouped as anti-helminthics, anti-fungal, anti-algal, anti- bacterial, anti-viral, anti-cancer and anti-diabetic. Docking study have been conducted for anti-helminthic compound with *M. incognita* targets such as beta 1, 4 – endoglucanases, glutathione S-transferase, chorishmatemutase and SOD to assess the inhibitory activity. Betatubulin and mitogen activated protein kinase enzymes from *P. capsici* have been docked with antifungal compounds from *P. putida*.



Fig. 40. IISR sequence repository database

AGRICULTURAL KNOWLEDGE MANAGEMENT UNIT

AKMU facilitates the IT and ICT related activities of the institute and ensures uninterrupted internet connectivity to all divisions/sections and guest houses. The repair and maintenance of computers, printers and computer accessories of various divisions/sections is facilitated through AKMU. The Personnel Management Information System Network – II (PERMISnet II) and Project Information & Management System of ICAR (PIMS-ICAR) is updated by AKMU. Adding new features in the office automation software (ARISoft) and modification and updation of Institute website, intranet portal, library portal, AICRPS website etc. were also done by AKMU. Apart from this AKMU assisted in statistical analysis of scientific data using SAS and other statistical software. Under the NAIP Subproject 'Strengthening of information & Communication Technology facility at ICAR institutes', new computers (25 Nos.), laser printers (5 Nos.), UPSs (5 Nos.), LCD projectors (2 Nos.), Extension kit and networking items were procured and installed at IISR Kozhikode, CRC Appangala, KVK and IISR Experimental Farm, Peruvannamuzhi. Optic fiber connectivity was established between the main building and Bio-control lab as well as guest houses.

Spicepedia

Spicepedia is a knowledge management platform exclusively for spices and related crops (Fig. 41). It is an adaptation of agropedia, which is an online platform developed by IIT, Kanpur. This Wiki-style platform provides a space for stakeholder interaction, best practice sharing, news updates, and certified contents. Knowledge objects loaded into the platform arranges itself into a web of relationships which is automatically computed. Variety of such features makes it easy for the users to access the information which would have been hitherto hidden. The platform enables sharing of information in Hindi, English, Malayalam and other regional languages. The credibility of the information provided is maintained by controlling the unauthorized usage by, allowing only registered users to upload content which can be reviewed by the editor. Till date 210 knowledge objects have been uploaded in Spicepedia which includes image, video and audio files. Technology transfer from lab to land can be made possible by this excellent platform.

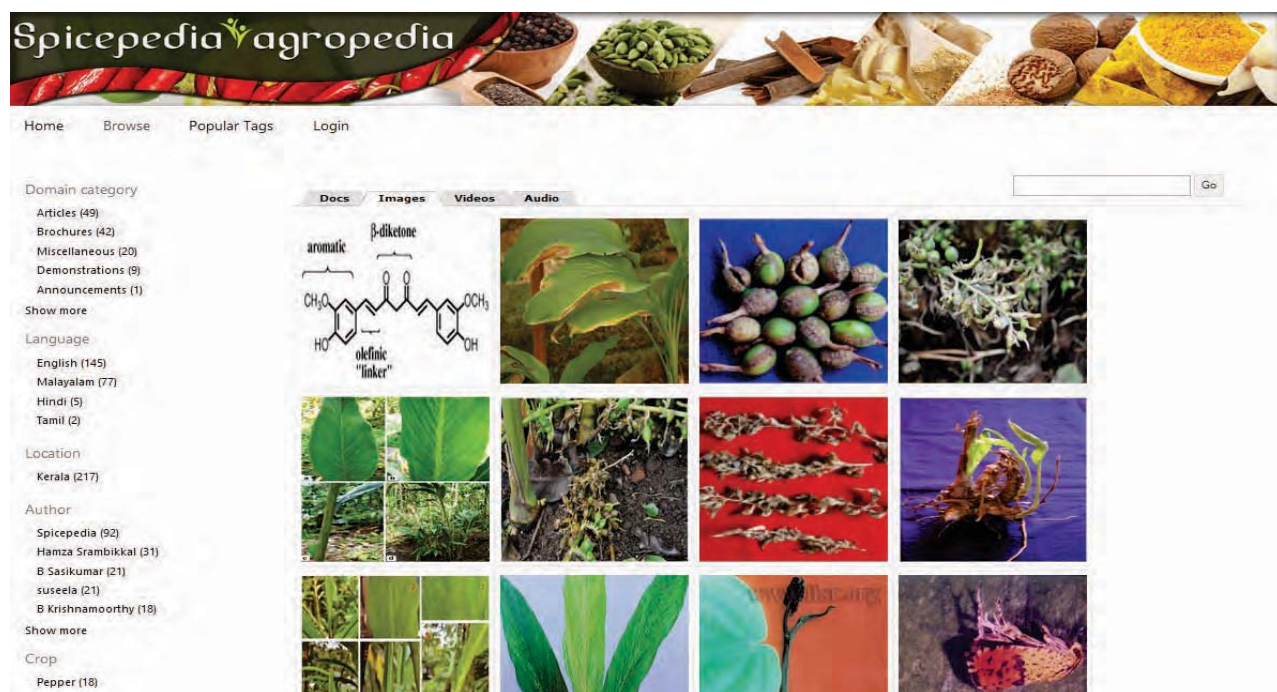


Fig. 41. Spicepedia a knowledge management platform for spices

NATIONAL INFORMATICS CENTRE FOR SPICES

New tools/portals

IISR Library has enabled web-scale discovery services (WDS) for its end users by implementing EBSCO Discovery Service (EDS). The library website was updated with a window for EDS and newly added information resources (Fig. 42). An online alert service called 'Latest in Spice Research' was developed and launched in the IISR intranet for updating the spice workers on the latest research article published globally on 12 mandate spice crops of IISR.

eGranth

IISR library became a member of NAIP e-Granth sub project “Strengthening of digital library and information management under NARS”. Under the NAIP eGranth project, the catalogue data of the entire library holding consisting of books, bound volumes of journals, technical reports, theses, project reports, reprints available in Libsyssoftware was converted into excel format and again modified into MARC 21 format after adding the additional required information. KOHA software Debian wheezy (7.0) was installed in the newly procured server and customized. The catalogue data converted into MARC 21 format was exported to KOHA and verified to check omissions.

The list of rare books published during and before 1960 was collected from the catalogue, prepared a list in excel sheet and in MARC 21 format. The books were digitized with the help of ANGRAU, AP and added to AGRI-KOSH. Similarly all the Institute publications that included Annual Reports, Research Highlights, newsletters, IRC Proceedings, Conference Proceedings, theses and priced and free publications were also digitized at ANGRAU. All the institutional publications, research articles etc. have been added to the open access institutional repository, DSpice, already developed at the Institute. The data were again added to Krishikosh for making it available for all libraries under NARS.

Additions to library resources/infrastructure

The Library subscribed CAB Direct online, 14 foreign journals and 53 Indian Journals and procured 71 reference books, 14 Wiley e-books and 9 technical reports, 6 theses/project reports during the year while 54 books were received on *gratis* base. The open source software Greenstone was updated with 10 new books which were scanned and converted to searchable e-books. Two new multifunction laser printers were purchased for the library. One server, two each new computers, laser printers and I KVA UPSs were added to the digital library. The back issues of journals (1500 Nos.) were bound.

Library continued to be a part of CeRA, the e-journal consortium of ICAR and catered to the requests from various CeRA members. The scope of digital institutional repository, DSpice, was widened with more institute publications. Twelve issues of the Agrititbits, the agricultural news service were brought out. During the year, there were 2035 computer users, 1235 internal users and 396 external users of the library facilities.



Fig. 42. The IISR library interface under the national eGranth network



AGRICULTURAL TECHNOLOGY INFORMATION CENTRE

EXTENSION AND TRAINING

Technology inputs

The three technology inputs distributed from the centre include quality planting material of improved varieties of spices, bio control agents and scientific publications including extension literature. Planting material for Rs. 202597, publications for Rs. 27199 and bio agents for Rs. 30961 were sold through ATIC generating a total income of Rs. 2, 61,257. The trend of component wise income generation during last five years through the centre is furnished in Fig. 43.

Farmer advisory services

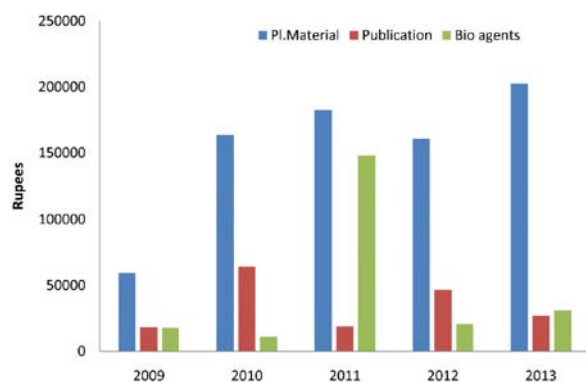


Fig. 43. Income generation form ATIC

During the year, 748 farmers availed advisory services through direct visit to ATIC; 546 of them were from Calicut; 102 from other districts of Kerala and 100 from other states (Fig. 44). Under ATMA or state sponsorship eight farmers group availed exposure training by study tour; five groups from Karnataka, three groups from Kerala. Four hundred and one students from various institutions in the country visited on study tour. 381 farmers availed information services through telephone calls and 115 farmers through letters.

Outreach extension

Institute participated in 10 Exhibitions /farmers meet. The main National level programmes included

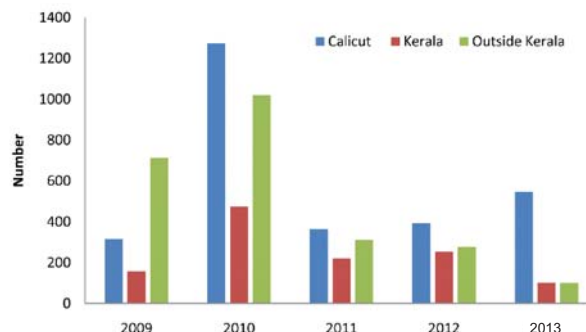


Fig. 44. Trends in farmers' visit to ATIC

krishsi vasant 2014 National Agricultural Fare at Nagpur and National Science Expo in connection with the 17th Kerala Science Congress at Kalpetta. The state level exhibitions included the Regional Agricultural fare at KAU, Trichur and Kerala State Flower show at Ambalavayal. Six Monthly technology Advisory services under the aegis of ATMA in Calicut district were conducted in which advisory services were provided to the department extension officials on a monthly basis. Four Front Line demonstrations of IISR Prathiba were laid out in Guntur district of AP to catalyse the adoption and spread of the variety already popular in the region.

Training and capacity building for extension functionaries and farmers

Under partnership services, the Institute offers training programme on demand from various agencies targeted for field extension functionaries of line departments, research workers of other ICAR Institutes and State Agricultural Universities (SAU). Courses are also offered for NGOs and private firms on request. The modules for these training programmes were prepared based on technologies developed by the institute and research achievements. The topics covered included spices production technology, nursery, pest and disease management in major spices and post harvest technology. During the year under report, three courses of five day duration on production

management and post harvest technology in spices were conducted for the officers for state Department of Agriculture/Horticulture of Uttarakhand, Assam and Arunachal Pradesh. Six monthly technology advisory services under the aegis of ATMA in Calicut district was conducted in which advisory services were provided based on reported field problems to the department extension officials on a monthly basis. A two day training course on scientific turmeric cultivation was carried out in Guntur district of Andhra Pradesh, in which 50 farmers from various districts of Andhra Pradesh attended.

Diffusion and impact studies

Sample survey and eight case studies of IISR Prathiba turmeric growers were carried out in Guntur district, Andhra Pradesh to study the spread of improved varieties and adoption of scientific cultivation practices. The study revealed that the variety IISR Prathiba has spread in 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 Dugiralla, Kadappa and Tekuurpet, the IISR Prathiba cultivators reported an average yield of 35 t ha⁻¹. The reported average yields of local cultivars were fluctuating and mean accounting to 20 t ha⁻¹. The perceived advantages of IISR Prathiba are shorter duration, high and stable yield, field tolerance to rhizome rot (rot is a recurrent problem in the area) and higher dry recovery of 22 % compared to 18 % for local. The critical technologies contributing to higher yield are organic amendments in the form of oil cakes and vermicompost and flood irrigation in dry months.

Technology showcasing

A technology showcasing was organized in collaboration with IISR and Indian Society for Spices at Hotel Crystal court Madikeri, Karnataka from Nov 27-29, 2013 (Fig. 45). Around 30 stalls including agricultural institutes and research organizations like IISR, Kozhikode; KVK, Peruvannamuzhi; Central Horticultural Experimental Station (CHES) Chettalli; Central Plantation Crops Research Institute (CPCRI),

Kasaragod; Directorate of Cashew Research (DCR) Puttur; Coffee Board, Bangalore; Krishi Vigyan Kendra, Gonikopal; Directorate of Areacanut and Spices Development (DASD), Calicut; Department of Horticulture, Madikeri; National Horticultural Research and Development Foundation (NHRDF) Nasik; CMFRI, Kochi; CIFT, Kochi; CTCRI, Thiruvananthapuram etc exhibited various agriculture related technologies. IISR also Participated in various exhibitions organized by ICAR institutes and government agencies.

Dissemination of innovative technology in print media

More than 45 news clippings (English/ Malayalam /Hindi/Tamil/Kannada), 13 Success stories appeared in various English/ Malayalam/ Hindi newspapers/agriculture magazines/portals and more than 20 items on ICAR/IISR websites and other language news portals. More than 15 TV news clippings in various English & Kannada TV channels appeared this year. Eight radio programmes including talks and interviews through the All India Radio, Kozhikode and Four talks/interviews were recorded and broadcast through Janavani FM, Kannur.



Fig. 45. Dr M Anandaraj, Director IISR inaugurating the stall during the exhibition at Madikeri

KRISHI VIGYAN KENDRA

Training programmes

The Kendra conducted 151 training programmes for practising farmers and farm women, rural youth and extension functionaries in the disciplines of agronomy, horticulture, animal sciences, home science, fisheries, plant protection and allied fields. A total of 5139 trainees were benefited out of the programmes (Fig. 46).



Fig. 46. Seminar on meliponiculture

FLD programmes

Eleven FLD programmes were undertaken to demonstrate new crop production and protection technologies in farmers' fields under different agro-climatic regions and farming situations (Fig. 47).

- ◆ Demonstration of shade tolerant high yielding variety of black pepper
- ◆ Popularization of bush pepper production technology
- ◆ Introduction of high yielding short duration upland rice variety Vaisakh
- ◆ Integrated disease management of *Phytophthora* foot rot of black pepper
- ◆ Popularization of bucket bio filter in maintaining water quality of ornamental fish culture tanks
- ◆ Popularization of grass carp for controlling submerged aquatic weeds

- ◆ High density planting of tissue culture nendran banana
- ◆ Demonstration of *Gramasree* layer chicks
- ◆ Introduction of a high yielding variety of amaranthus (Renusree)
- ◆ Value added product of spices and coconut inflorescence with branding and marketing
- ◆ Formulation of homemade rations for livestock and fishery



Fig. 47. FLD on bush pepper

OFT programmes

These programmes aim at testing the new technologies developed at research stations to ensure their suitability and sustainability to the specific locations and to suggest or modify or refine the technology accordingly. This was done by testing a released technology in real farm situation with the participation of farmer. The major OFT programmes carried out during the period are listed below:

- ◆ Assessing the performance of Arka Kalyan and Agrifound Dark Red onion under Kozhikode condition
- ◆ Assessing the performance of yard long bean varieties Lola, Vellayani Jyothika and Arka Mangala in Kozhikode district
- ◆ Performance evaluation of sandless nursery mixture for black pepper serpentine nursery



- ◆ Pest and disease management of bitter gourd under organic cultivation
- ◆ Management of *phytophthora* foot rot of black pepper (continuing)
- ◆ Evaluating the growth performance of fishes using different feeds

Sponsored training programme - “Gardeners training”

Two gardeners' training programmes of six months duration were organized under the sponsorship of State Horticulture Mission empowering 50 rural youth. The programme comprised of “on hand” practical training on all aspects of planting material production, garden maintenance and production technology of major crops. Study tour programmes were also organized to various research institutes, farms, nursery and progressive farmers' fields.

Revolving fund programme

The Kendra has a strong revolving fund programme to generate income for productive uses. Under this programme, quality planting materials of various crops are produced and made available to public at affordable rates. Also, income was generated by way of sale of layer chicks, goats, heifers and bulls and consultation and doorstep services through the clinic. During the period, an amount of Rs.17.85 lakhs has been realised through sale of planting materials, bioproducts, bioagents and the activities of Plant and Animal Health Centre.

Plant and animal health centre

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

Consultancy/advisory/home service carried out -676

Artificial insemination carried out : 179
 No. of Animal health campaigns/infertility camps: 2
 Vaccination of poultry birds and animals: 41,200
 Block *ksheeroltsavam*: 4

Other extension activities

The Kendra conducted 15 seminars, participated in 12 Kisan Mela cum exhibitions, broadcasted four radio talks and conducted three study tour for farmers to various research institutes.

Demonstration units

The following demonstration units are maintained by the KVK.

- ◆ Medicinal plant unit
- ◆ Model homestead garden
- ◆ Model arecanut seed garden
- ◆ Guava demonstration unit
- ◆ Sapota demonstration unit
- ◆ Vermiculture unit
- ◆ Nutmeg scion bank
- ◆ Dairy unit
- ◆ Goatary unit
- ◆ Layer unit
- ◆ Broiler unit
- ◆ Ornamental fish culture unit
- ◆ Anthurium unit
- ◆ Pot culture of vegetables
- ◆ Coconut nursery

Kisan mobile SMS service

KVK started short message Service (SMS) to all registered farmers on latest updates in agriculture and allied fields over their mobile phones. The SMS are being sent to farmers regarding new interventions, latest technologies, market price of agriculture produce, weather forecast, disease management measures, planting material availability forthcoming trainings etc. KVK has so far sent 32 SMS, 13 voice messages benefitting 743 farmers and 100 Extension functionaries.

Technology week celebration during 2013-14

Technology week (*Vithum Kaikkottum*) of the Kendra was also conducted from 21st to 24th January 2014 in which about 150 persons including farmers, extension functionaries, and school children were attended. Experience sharing of farmers, seminars, exhibition, sale of techno inputs, method demonstrations, quiz and elocution competitions for school children were also organized as part of the celebration (Fig. 48).

Award for KVK beneficiaries

Three farmers/farmer groups received awards from National Institutions during the period in recognition of their achievements in the field of agriculture.



Fig. 48. Inauguration of technology week at KVK, Peruvannamuzhi



RESEARCH PUBLICATIONS

1. Abirami K, Rema J & Mathew P A 2012 Evaluation of nutmeg genotypes for seed germination, seedling growth and graft take. *J. Medicinal Aromatic Plants* 34: 91-94.
2. Anandaraj M, Prasath D, Kandiannan K, John Zachariah T, Srinivasan V, Jha A K, Singh B K, Singh A K, Pandey V P, Singh S P, Shoba N, Jana J C, Ravindra Kumar K & Uma Maheswari K 2014 Genotype by environment interaction effects on yield and curcumin in turmeric (*Curcuma longa* L.). *Indl. Crops Products* 53: 358–364.
3. Anoop K & Suseela Bhai R 2014 Evaluation of antagonistic potential of indigenous *Trichoderma* isolates against *Pythium aphanidermatum* (Edson) Fitz. causing rhizome rot in turmeric (*Curcuma longa* L.). *J. Sci.* 4: 99–105.
4. Anoop K & Suseela Bhai R 2013 Host range study of turmeric rhizome rot pathogen *Pythium aphanidermatum* on selected Zingiberaceae members. *Intl. J. Res. Pure Appl. Microbiol.* 3: 113–115.
5. Barnwal P, Singh K K, Kumar R & Zachariah T J 2013 Thermal properties of cryo-ground powder of black pepper (Panniyur 1). *J. Spices Arom. Crops* 22: 148–153.
6. Bhat A I & Siljo A 2014 Detection of viruses infecting black pepper by SYBR Green-based real-time PCR assay. *J. Plant Path.* 96: 105–109.
7. Bhat A I, Siljo A & Deeshma K P 2013 Rapid detection of *Piper yellow mottle virus* and *Cucumber mosaic virus* infecting black pepper (*Piper nigrum*) by loop-mediated isothermal amplification (LAMP). *J. Virological Methods* 193: 190–196.
8. Biju C N, Praveena R, Ankegowda S J, Darshana C N & Jashmi K C 2013 Epidemiological studies on black pepper anthracnose caused by *Colletotrichum gloeosporioides*. *Indian J. Agril. Sci.* 83: 1199–1204.
9. Deepa K, Sheeja T E, Santhi R, Sasikumar B, Anu Cyriac, Deepesh P V & Prasath D 2013 A simple and efficient protocol for isolation of high quality functional RNA from different tissues of turmeric (*Curcuma longa* L.). *Physiol. Mol. Biol. Plants* 20(2): 263–271.
10. Devashayam S, Jacob T K, Abdulla Koya K M, Sasikumar B and Prasath D 2011 Screening turmeric (*C. longa* L.) germplasm for resistance to shoot borer (*Cognothes punctiferalis* Guen) (Lepidoptera:Pyralidae) in Kerala, South India. *Entomon* 36(1-4): 59-62.
11. Dinesh R & Ghoshal Chaudhuri S 2013 Soil biochemical/microbial indices as ecological indicators of land use change in mangrove forests. *Ecol. Indic.* 32: 253–258.
12. Dinesh R, Anandaraj M, Kumar A, Srinivasan V, Bini Y K, Subila K P & Aravind R 2013 Effects of plant growth promoting rhizobacteria and NPK fertilizers on biochemical and microbial properties of soils under ginger (*Zingiber officinale*) cultivation. *Agril. Res.* 2: 346–353.
13. Dinesh R, Srinivasan V, Hamza S & Anandaraj M 2014 Massive phosphorus accumulation in soils: Kerala's continuing conundrum. *Curr. Sci.* 106: 343–344.
14. Jayashree E & Visvanathan R 2013 Development of a hand operated diamond cut mesh drum abrasive ginger peeler. *J. Spices Arom. Crops* 22: 174–180.
15. Jayashree E & Visvanathan R 2013 Mathematical modeling for thin layer drying of ginger (*Zingiber officinale* Rosc.). *J. Spices Arom. Crops* 22: 24–30.
16. Jayashree E & Visvanathan R 2013 Studies on thin layer drying characteristic of ginger (*Zingiber officinale* Rosc.) in a mechanical drier. *J. Plantn. Crops* 41: 86–90.
17. Jayashree E, John Zachariah T, Evangelin F P P & Suseela Bhai R 2012 Qualitative changes during storage of different ginger based spice sauces. *J. Hortl. Sci.* 7: 174–179.
18. Krishnamurthy K S, Ankegowda S J, Srinivasan V & Hamza S 2013 Influence of carbohydrates,



- mineral nutrients and plant hormones in alternate bearing of black pepper (*Piper nigrum* L.) Am. J. Plant Sci. 4: 1960–1967.
19. Krishnamurthy K S, Diggs A & Porterfield D M 2013 Amperometric biosensor approaches for quantification of indole 3-acetic acid in plant stress responses. Commun. Soil Sci. Plant Anal. 44: 1749–1763.
 20. Kumar A, Prameela T P & Suseelabhai R 2013 A unique DNA repair and recombination gene (recN) sequence for identification and intraspecific molecular typing of bacterial wilt pathogen *Ralstonia solanacearum* and its comparative analysis with ribosomal DNA sequences. J. Biosci. 38: 267–278.
 21. Kumar A, Prameela T P, Bhai R S, Siljo A, Anandaraj M & Vinatzer B A 2014 Host specificity and genetic diversity of race 4 strains of *Ralstonia solanacearum*. DOI: 10.1111/ppa.12189 Plant Pathology.
 22. Nileena C R, Srinivasan V, Hamza S, Leela N K & Thankamani C K 2014 Quality profile of ginger under different management systems. J. Spices Arom. Crops 23: 125–129.
 23. Parvathy V A, Swetha V P, Sheeja T E and Sasikumar B 2014 DNA barcoding to detect chilli adulteration in traded black pepper powder. Food Biotech 28: 25-40.
 24. Pervez R, Devasahayam S, Eapen S J & Jacob T K 2014 Determination of LD50 and LT50 of entomopathogenic nematodes against shoot borer (*Conogethes punctiferalis* Guen.) infesting ginger. Ann. Plant Prot. Sci. 22: 169–173.
 25. Pervez R, Jacob T K, Devasahayam S & Eapen S J 2014 Penetration and infectivity of entomopathogenic nematodes against *Lema* sp. infesting turmeric. J. Spices Arom. Crops 23: 71–75.
 26. Prasath D, Suraby E J, Karthika R, Rosana O B, Prameela T P & Anandaraj M 2013 Analysis of differentially expressed genes in *Curcuma amada* and *Zingiber officinale* upon infection with *Ralstonia solanacearum* by suppression subtractive hybridization. Acta Physiologiae Plantarum DOI: 10.1007/s11738-013-1362-2.
 27. Praveena R, Biju C N, Senthil Kumar R, Darshana C N & Jashmi K C 2013 Preliminary evaluation of cardamom accessions against leaf blight/Chenthal disease. Indian Phytopathol. 66: 112–113.
 28. Rosana O B, Krishna P B & Eapen S J 2014 Virtual screening and *in vitro* assay to explore novel inhibitors from black pepper against potential targets of *Radopholus similis*. Intl. J. Comp. Appl. 86: 35–43.
 29. Sheeja T E, Anju P R, Shalini R S, Siju S, Dhanya K & Krishnamoorthy B 2013 RAPD, SCAR and conserved 18S rDNA markers for a red-listed and endemic medicinal plant species, *Knema andamanica* (Myristicaceae). Physiol. Mol. Biol. Plants 19: 245–250.
 30. Sheeja T E, Hima Bindu K, Anto P, Dhanya K, Siju S & Vasantha Kumar T 2013 A SCAR marker based method for sex determination in dioecious betel vine (*Piper betle*). Indian J. Agril. Sci. 83: 1409–10.
 31. Sheeja T E, Rosana O B, Swetha V P, Shalini R S, Siju S, Dhanya R, Rahul P R & Krishnamoorthy B 2013 The 18S rDNA gene discriminates between red-listed and unexplored ethnomedicinal species of *Myristicaceae* restricted to humid tropics of India. Genet. Resour. Crop Evol. 61:523-535.
 32. Sheeja T E, Sabeesh C, Shabna O V, Shalini R S & Krishnamoorthy B 2013 Genetic diversity analysis of *Myristica* and related genera using RAPD and SSR markers. J. Spices Arom. Crops 22: 38–46.
 33. Sheeja T E, Uma G, Sasikumar B, Saji K V & Rahul P R 2013 Genetic diversity study in *Piper* spp. using inter simple sequence repeat (ISSR) markers. J. Spices Arom. Crops 22: 111–119.
 34. Shiva K N, Zachariah T J, Leela N K & Mathew P A 2013 Performance of paprika and paprika alike chillies (*Capsicum annum* L.) under warm humid tropics. J. Spices Arom. Crops 22: 222–227.
 35. Siju S, Dhanya K, Sasikumar B & Sheeja T E 2014 Methods for development of microsatellite markers: An overview. Not Sci. Biol. 6: 1–13.

- 36..Siju S, Dhanya K, Sheeja T E, Sasikumar B, Bhat A I & Parthasarathy V A 2013 Novel polymorphic microsatellite markers from turmeric, *Curcuma longa* L. (*Zingiberaceae*). *Acta Bot. Croat. J.* 72: 407–412.
- 37.Siljo A & Bhat AI 2014 Reverse transcription loop-mediated isothermal amplification assay for rapid and sensitive detection of *Banana bract mosaic virus* in cardamom (*Elettaria cardamomum*). *Europ. J. Plant Path.* 138: 209–214.
- 38.Siljo A, Bhat A I & Biju C N 2013 Symptomatology and coat protein gene sequence studies suggest high variability in *Cardamom mosaic virus* isolates occurring in India. *J. Spices Arom. Crops* 22: 70–75.
- 39.Siljo A, Bhat A I & Biju C N 2014 Detection of *Cardamom mosaic virus* and *Banana bract mosaic virus* in cardamom using SYBR Green based reverse transcription-quantitative PCR. *Virus Dis.* (formerly *Indian J. Virol.*) 25: 137–141.
- 40.Sinoj J, Sheeja T E, Bhai R S, Suraby E J, Minoo D, Soji J, Jayakumar V N, Anandaraj M, Bansal K C & Nirmal Babu K 2014 Somatic embryogenesis and transgenic development in black pepper for delayed infection and decreased spread of foot rot caused by *Phytophthora capsici*. *J. Plantn Crops* 42:11-19.
- 41.Srinivasan V, Kandiannan K & Hamza S 2013 Efficiency of sulphate of potash (SOP) as an alternate source of potassium for black pepper (*Piper nigrum* L.). *J. Spices Arom. Crops* 22: 120–126.
- 42.Sruthi D, John Zachariah T, Leela N K & Jayarajan K 2013 Correlation between chemical profiles of black pepper (*Piper nigrum* L.) var. Panniyur-1 collected from different locations. *J. Med. Plants Res.* 7: 2349–2357.
- 43.Suseela Bhai R, Sasikumar B & Kumar A 2013 Evaluation of ginger germplasm for resistance to soft rot caused by *Pythium myriotylum*. *Indian Phytopath.* 66: 93-95.
- 44.Swetha V P, Parvathy V A, Sheeja T E & Sasikumar B 2013 Isolation and amplification of genomic DNA from barks of *Cinnamomum* spp. *Turk. J. Biol.* 37: DOI:10.3906/biy-1308-5.
- 45.Utpala P, Nirmal Babu K, Senthil Kumar R, Ashis G R, Mohan S & Parthasarathy V A 2013 Diversity of Indian *garcinia* - a medicinally important spice crop in India. *Acta Hort.* 979: 467–476.
- 46.Utpala P, Nandakishore OP, Nirmal Babu K & Senthil Kumar R 2013 Comparative effectiveness of inter-simple sequence repeat and randomly amplified polymorphic DNA markers to study genetic diversity of Indian *Garcinia*. *Afr. J. Biotech.* 12: 443–451.
- 47.Utpala P, Asish G R, Saji K V, Johnson G K, Leela N K & Mathew P A 2014 Diversity study of leaf volatile oil constituent of Piper species based on GC/MS and spatial distribution. *J. Spices & Arom. Crops* 23: 10–16.
- 48.Vijesh Kumar I P, Reena N, Anandaraj M, Eapen S J, Johnson G K & Vinitha K B 2013 Amplification, cloning and in silico prediction of full length elicitor gene from *Phytophthora capsici*, the causal agent of foot rot disease of black pepper. *J. Plant Pathol. Microb.* 4: 181.

EDUCATION AND TRAINING

Trainings/workshops conducted

- ◆ Workshop on issues related to pesticide residues in spices, 03 June 2013
- ◆ Workshop on adoption of agropedia platform for spices crops, 07 June 2013
- ◆ Training programme on information literacy in the digital era, 12 August 2013
- ◆ Launch workshop on business planning and development unit, 21 September 2013
- ◆ Entrepreneur development programme in spices, 06 December 2013
- ◆ Workshop on intellectual property and its management for growth and prosperity, 16 January 2014
- ◆ Interactive meeting on advances in biosciences, 26 February 2014
- ◆ National Workshop on developing institutional repositories using DSpice, 12-13 March 2014
- ◆ Training programme on next generation sequencing: data analysis and annotation, 17-20 March 2014 (Fig. 49).

M.Sc. / Ph.Ds

- ◆ One student was awarded with Ph.D., from Mangalore University



Fig. 49. Training programme on next generation sequencing: data analysis and annotation

BUSINESS PLANNING AND DEVELOPMENT - INSTITUTE TECHNOLOGY MANAGEMENT UNIT

The total sanctioned budget for the BPD unit was Rs.115.50 lakhs for a period of one year. The total expenditure was 103.35 lakhs. The office and incubation facility have been created for housing five incubatees at a time. The technological highlight is the establishment of a processing facility equipped with state of art facilities for both wet and dry processing of spices comprising of about 20 equipments. The facility was designed in conformation with the ISO standards and the staffs are being trained on various aspects of quality management and food safety standard systems through approved consultants. The BPD unit is also equipped with a high-tech solar drying yard and a nursery hardening facility for disease free planting material production.

The unit has organized Entrepreneurship Development Programmes, IPR awareness workshop and business meet, which invited a tremendous response from more than 300 farmers and entrepreneurs (Fig. 50). Eight entrepreneurs have enrolled with BPD and 12 were supported. Eight consultancy assignments were undertaken and linkages have been established with 14 organizations. A BPD advisory board was constituted under the chairmanship of the Director. Technology commercialization and patent protection has been

initiated through National Research Development Corporation (NRDC). The BPD unit has filed six patents for a seed coating composition, a biocapsule technology and four micronutrient compositions to entrepreneurs and two companies viz., Natura Nursery & Agroproducts and Hi 7 Agri Bio Solutions have procured the micronutrient mixture technology for turmeric and ginger. One non-exclusive license was issued for ginger variety, IISR Varada. The nutmeg variety, IISR Keralaashree credited as the first variety developed through a farmer's participatory approach is under process of Memorandum of Agreement with IISR with licensing rights delegated to the farmer and IP rights delegated to IISR and farmer combined. The total revenue generated including licensing fee & royalties, consultancy services, BPD Unit membership fee, contract services and training fee is Rs.11.67 lakhs. One popular article was published in Spice India on non-exclusive licensing of technologies and five media releases on BPD were made. One brochure on BPD and one folder on IISR technologies were published and distributed among spice growers and entrepreneurs. The unit has participated in eight seminars/symposiums/ workshops for showcasing IISR technologies (Fig.51).



Fig. 50. Inauguration of launch workshop on business planning and development unit



Fig. 51. Technology showcasing at Madikeri, Karnataka.

HINDI CELL ACTIVITIES

Official Language Implementation Committee (OLIC)

The OLIC met once in every quarter (20th June 2013; 31st October 2013; 7th November 2013 and 3rd March 2014) under the chairmanship of Dr. M. Anandaraj, Director the official language implementation activities of the institutes.

Workshop

Three Hindi workshops (noting and drafting in official language on 14th June 2013; rules of official language on 25th September 2013; Hindi translation and words pronunciation on 9th January 2014) were organized to popularize the official language.

Hindi day and Hindi fortnight celebration

Hindi day (14th September 2013) and Hindi fortnight (23rd September 2013 to 5th October 2013) were celebrated. Hindi fortnight was inaugurated on 23rd September 2013 by Dr. M. Anandaraj, Director, IISR, Kozhikode (Fig. 52). During the week various competitions viz., extempore speech, song, debate, noting and drafting, memory test, caption writing and anthakshari were conducted for the staff members and prizes were distributed to the winners in the valedictory function held on 5th October 2013. Dr. Jagdeep Saxena, I/C Hindi unit, DKMA, ICAR, New Delhi was the chief guest. Institute official language magazine, *Masaloon ki Mehak* was also released on this occasion.

TOLIC activity

Dr TJ Zachariah, Head, Division of Crop Production and Post Harvest Technology, Dr. Rashid Pervez, Senior Scientist and Hindi Officer and Ms. N. Prasannakumari, Hindi Translator attended the half yearly TOLIC meeting at Malabar Palace Kozhikode on 6th March 2014.

Dr. Rashid Pervez and Ms. N. Prasannakumari attended the subcommittee meeting of TOLIC at SBT, Kozhikode on 12th July 2013 and 6th January 2014 and Dr. Rashid Pervez participated in the Hindi workshop on 19th December, 2013 at BSNL conference hall, Kozhikode.

OL implementation Inspection

Dr. Sheela, Deputy Director (OL), CMFRI, Kochi inspected official language implementation activities in the institute on 7th November 2013.

Publications (2013-14)

- ◆ Annual report (2012-13)
- ◆ *Anusandhan ke mukhya ansh* (2012-13)
- ◆ *Masala Samachar* (4 issues)
- ◆ *Rashtrya ki unnatee mein Masaloon ka yogdan*
- ◆ *Masaloon ki Mehak* (OL magazine)
- ◆ Bulletins (*Kalimirch, Haldi* and *Adrak*)

Official Language reports

Quarterly and annual reports on official language activities of the institutes were prepared and sent to ICAR, New Delhi; TOLIC, Kozhikode and Regional Implementation Office, Kochi. The half yearly report on official language implementation was prepared and submitted to Regional Implementation Office, Kochi.

Other activities

Translated various item under 3(3) viz., office order, circular, documentaries, rubber stamps, name board, envelop and web site into Hindi. Displayed daily a word/phrase in hindi and its transliteration in English.



Fig. 52. Hindi fortnight valedictory function

RECOGNITION

The Institute was awarded Rajbhasha Shield 2013, selected from among the 70 central government

organizations of Kozhikode in the annual meeting of the TOLIC on 6th March 2014 (Fig. 53).



Fig. 53. Rajbhasha Shield award 2013 received by Dr TJ Zachariah, Head, Division of Crop Production and Dr Rashid Pervez, Senior Scientist & Hindi Officer



INSTITUTE MANAGEMENT COMMITTEE

Dr. M. Anandaraj, Director Indian Institute of Spices Research, Kozhikode, Kerala	Chairman
Director of Agriculture, Govt. of Kerala, Thiruvananthapuram, Kerala	Member
Director of Horticulture Govt. of Tamil Nadu, Chennai, Tamil Nadu	Member
Dr. B. Raju , Vice-Chancellor, University of Horticulture Sciences, Bagalkot, Karnataka	Member
Sri. A.V. Joseph, Chief Finance & Accounts Officer CMFRI, Kochi, Kerala	Member
Dr. R. Dhanapal, Principal Scientist, Sugarcane Breeding Institute, Coimbatore, Tamil Nadu	Member
Dr. J. Rema, Principal Scientist Indian Institute of Spices Research, Kozhikode, Kerala	Member
Dr. A.T. Sadashiva, Principal Scientist, Division of Vegetable Crops, Indian Institute of Horticulture Research, Bengaluru, Karnataka	Member
Dr. S.D. Sawant, Director, Nation Research Centre for Grapes, Pune, Maharashtra	Member
Dr. Suresh Kumar Malhotra, Asst. Director General (Hort.II) Indian Council of Agricultural Research, New Delhi	Member
Administrative Officer Indian Institute of Spices Research, Kozhikode, Kerala	Member Secretary
Sri. Sulfikar Mayoore Mayoore, Vaidyar Veedu, Kayamkulam, Alappuzha, Kerala	Non-official Member
Sri. Adv. C.V. Damodharan Kattukulangara, Anandashram, Kanhangad, Kasaragod, Kerala	Non-official Member

LIST OF PROJECTS

I. Institute Projects:

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

1. Gen. X (813): Breeding cardamom for high yield and disease resistance (2007-2015) [R Senthil Kumar, R Praveena and C M Senthil Kumar]
2. Gen. XXIX (813): A comparative study of molecular and bio-chemical diversity of *Garcinia* of Eastern Himalayas and Western Ghat ranges with GIS (2008-2014) [Utpala Parthasarathy and R Senthil Kumar]
3. Gen. XXXV (813): Genetic improvement in turmeric through seedling selection and hybridization (2013-2020) [Dr. R. Ramakrishnan Nair and Mr. B. Krishnamoorthy]
4. Gen. XXXI (813): Breeding black pepper for high yield, quality and resistance to stresses (2012-2017) [B Sasikumar, Johnson K George, K V Saji, T E Sheeja, T John Zachariah, R Suseela Bhai, K S Krishnamurthy and S Devasahayam]
5. Gen. XXXII (813): Expression profiling and allele mining of genes induced under water-deficit stress in black pepper (2012-15) [Johnson K George and K S Krishnamurthy]
6. Gen. XXXIV (813): Induction of variability in ginger through induced mutation for yield and disease resistance (2012-2017) [D Prasath, R Ramakrishna Nair and R Suseela Bhai]
7. Gen. XXX (813): Evaluation of genetic variability in vanilla with emphasis to disease tolerance (2010-2015) [R Ramakrishnan Nair]
8. Biotech. XII (813): Mining of DNA markers and genes from expressed sequence tags of *Curcuma longa* (2012-2015) [Sheeja TE and B Sasikumar]

Mega Project II: Collection, conservation, characterization and cataloguing of germplasm of

Spice crops for yield and other economically important characters [Project Leader: K V Saji]

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

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

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

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

1. SSC VI (813): Nutrient cycling and soil C sequestering potential of spice crops under different management systems (2011-2015) [V Srinivasan, R Dinesh, S J Ankegowda and S Hamza]
2. Agr. XXIX (813): Effect of weed management practices on growth, yield and quality parameters of ginger (2011-2014) [C K Thankamani and K Kandiannan]



- Hort. VII (813): Evaluation of nutmeg for its suitability for high density planting (2011-2016) [J Rema and R Senthil Kumar]

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

- PHT. VI (813): Studies on production of food extrudates from selected spices (2011-14) [E Jayashree, T John Zachariah and Thajudeen Sheriff(CTCRI)]
- Biochem. VIII (813): Evaluation of spice extracts for anticancer effect in relation to telomerase activity (2012-2016) [B Chempakam and K Sujathan (RCC, Thiruvananthapuram)]
- PHT VII (813): Developing energy efficient processing technologies for spices (2013-2017) [E Jayashree and NK Leela]
- Org. Chem. IV (813): Chemoprofiling of *Myristica* spices for nutraceutical and medicinal properties (2013-2018) [N K Leela, T John Zacharia and, B Chempakam]

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

- ICAR Mega Seed Project: Production of nucleus planting materials of improved varieties of spice crops (2006-2017) [K Kandiannan, P A Mathew and S J Ankegowda]

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

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

- Path. XX (813): Screening of *Piper* germplasm accessions against *Piper yellow mottle virus* (PYMoV) (2008-2015) [A Ishwara Bhat, T K Jacob and K V Saji]
- Nema. IV (813): Role of phenyl propanoids in black pepper - burrowing nematode interactions (2008-2014) [Santhosh J Eapen and Johnson K George]

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

- Crop. Prot. 1.5 (813): Integrated management of *Phytophthora* foot rot and slow decline diseases of black pepper (2008-2014) [R Suseela Bhai, Santhosh J Eapen and Rashid Pervez]
- Nema. VI (813): Mass production and field evaluation of promising entomopathogenic nematodes against insect pests infesting major spices (2012-2016) [Rashid Pervez, Santhosh J Eapen and S Devasahayam]
- Path. XXI (813): Diversity of rhizome – root rot pathogens and their antagonists in cardamom. (2010 – 2014) [R Praveena and C N Biju]
- Path XXII (813): Investigations on the endophytic and rhizospheric microflora associated with cardamom and allied genera (2012-15) [C N Biju and R Praveena]
- Ent. XIV (813): Survey and documentation of naturally occurring entomopathogens in spice cropping systems (2012-2015) [C M Senthil Kumar, T K Jacob and S Devasahayam]

Mega Project X: Transfer of Technology and Impact Assessment [Project Leader: P. Rajeev]

- Ext. IV(813) : Training of Research and Extension Personnel (2005-2014) [P Rajeev]
- Ext. V (813): A Study on Diffusion, Adoption and Impact of Varieties released from IISR and Scientific Crop Management Practices (2006-14) [P Rajeev]

Mega Project XI: Developing Customized Software and Expert-System on Spices [Project Leader: S J Eapen]

- Agr XXX (813): Database on Agro-technologies Generated for Major Spices (Black pepper, Cardamom, Ginger and Turmeric) in Agro-climatic Regions of India (2011 -2014) [K Kandiannan, Utpala Parthasarathy, K Jayarajan and CK Sushamadevi]
- Ext. VI (813): Spicepedia – A knowledge base for spices (2013-2015) [P Rajeev and K Jayarajan]



II. Externally aided Projects:

i) Department of Biotechnology, New Delhi

1. DBT Twinning Programme for the NE: Seed system development in major spice crops (Ginger, turmeric and Naga Chilli) of NER through *in vitro* techniques (2012-2015) [K Nirmal Babu and K. Kandiannan]
2. DBT-CIB-5: Development of comprehensive SSR and SNP markers for the study of genetic diversity and association analysis in *Curcuma* (2012-2015) [T E Sheeja, D Prasath and B Sasikumar]
3. DBT-CP6: Genome mining of spice associated endophytic bacteria for natural products (2011-2014) [Santhosh J Eapen and R Suseela Bhai]
4. DBT-CP5: Testing transgenic black pepper for resistance to viruses (2011-2014) [A. Ishwara Bhat and D. Prasath]
5. DBT-SS1: Distributed Information Sub-Centre (2000-2017) [Santhosh J Eapen]

ii) Indian Council of Agricultural Research, New Delhi

1. ICAR-CPPHT-1: Network Project on Organic Farming (2013-2017) [C K Thankamani, V Srinivasan and T John Zachariah]
2. ICAR-CP 4: Application of Microorganisms for Agriculture and Allied Sectors (AMAAS): Nutrient management, PGPR and biocontrol (2007-2014) [M Anandaraj, R Dinesh and N K Leela]
3. Outreach Programme on *Phytophthora*, *Fusarium* & *Ralstonia* Diseases of Horticultural and Field Crops (2008-2015) [M Anandaraj, R Suseela Bhai, Santhosh J Eapen, K Nirmal Babu, Johnson K George, D Prasath and P Umadevi]
4. Outreach Programme on Management of sucking pests in Horticultural Crops: (2009-2015) [T K Jacob, S Devasahayam and C M Senthil Kumar]
5. Outreach Programme on Diagnosis and Management of Leaf Spot Diseases in Field and Horticultural Crops (2009-2015) [C N Biju and R Praveena]

iii) Ministry of Food Processing Industries, New Delhi

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

iv) Department of Information & Technology, New Delhi

1. DoE-CPPHT-1: Developing electronic nose for Monitoring Cardamom aroma (2012-14) [N K Leela and Nabarun Bhattacharya]

v) Department of Science and Technology, New Delhi

1. DST-CPPHT-1: Development of mechanical unit for production of white pepper from green pepper (2012-14) [E Jayashree, R Suseela Bhai, T John Zachariah and Ravindra Naik (RRL)]

vi) National Agricultural Innovation Project, New Delhi

1. NAIP-CPPHT-1: Studies on cryogenic grinding for retention of flavour and medicinal properties of some important Indian spices (2009-2014) [T John Zachariah and N K Leela]
2. NAIP SS-II: Mobilizing Mass Media Support for Sharing Agro-Information (2009-2014) [T J Zachariah, P Rajeev and T K Jacob]

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

1. Kerala State-CPPHT-1: Soil based nutrient management plan for agro ecosystems of Kerala (2010-2014) [R Dinesh, V Srinivasan and S Hamza]
2. Kerala State – CPPHT-2: Pepper Rehabilitation Package – Technology Mission on Black pepper for Wayanad – SUGANDHI (2010-2014) [V Srinivasan, T K Jacob, R Suseela Bhai, R Dinesh, C K Thankamani, K Kandiannan, A Ishwara Bhat, Santhosh J Eapen, S J Ankegowda, Rashid Pervez, K S Krishnamurthy, P Rajeev, C N Biju and S Hamza]
3. Kerala State – CPPHT-3: Integrated pepper research and development Project for North Kerala districts (2013-2016) [V. Srinivasan, P.S. Manoj, K.M. Prakash, K.K. Aiswariya, P. Rajeev, S. Hamza, R. Suseela Bhai, T.K. Jacob, A. Ishwara Bhat, Santhosh J. Eapen, Rashid Pervez, R. Dinesh, C.K. Thankamani, K. Kandiannan, K.S. Krishnamurthy, K.V. Saji]



PERSONNEL

Headquarters

Scientific

Name	Designation
Dr. M. Anandaraj	Director
Dr. K. Nirmal Babu	Project coordinator (Spices)
Dr. S. Devasahayam	Head, Crop Protection Division
Dr. T. John Zachariah	Head, Crop Production & PHT Division
Mr. B. Krishnamoorthy	Principal Scientist (Plant Breeding) (till 30.11.13)
Dr. B. Chempakam	Principal Scientist (Biochemistry) (till 28.02. 2014)
Dr. B. Sasikumar	Principal Scientist (Plant Breeding) & Head in charge CI & BT Division
Dr. T.K. Jacob	Principal Scientist (Entomology)
Dr. J. Rema	Principal Scientist (Horticulture)
Dr. Johnson K. George	Principal Scientist (Gen. & Cytogenetics)
Dr. C.K. Thankamani	Principal Scientist (Agronomy)
Dr. R. Dinesh	Principal Scientist (Soil Science)
Dr. R. Suseela Bhai	Principal Scientist (Plant Pathology)
Dr. A. Ishwara Bhat	Principal Scientist (Plant Pathology)
Dr. R. Ramakrishnan Nair	Principal Scientist (Gen. & Cytogenetics)
Dr. K.S. Krishnamurthy	Principal Scientist (Plant Physiology)
Dr. K. Kandiannan	Principal Scientist (Agronomy)
Dr. N.K. Leela	Principal Scientist (Org. Chemistry)
Dr. Santhosh J. Eapen	Principal Scientist (Nematology)
Dr. K.V. Saji	Principal Scientist (Economic Botany)
Dr. P. Rajeev	Principal Scientist (Agril. Extension)
Dr. V. Srinivasan	Principal Scientist (Soil Science)
Dr. A. Shamina	Sr. Scientist (Biochemistry-PS) – (till 31.12. 2013)
Dr. T.E. Sheeja	Senior Scientist (Biotechnology)
Dr. Rashid Pervez	Senior Scientist (Nematology)
Dr. D. Prasath	Senior Scientist (Horticulture)
Dr. C.M. Senthilkumar	Senior Scientist (Entomology)
Dr. E. Jayashree	Senior Scientist (AS & PE)
Ms. P. Uma Devi	Scientist (Biotechnology)

Technical Officers

Dr. Johny A. Kallapurackal	Technical Officer (T9) (till 30.06.2013)
Dr. Hamza Srambikkal	Chief Technical Officer (Lab) (T9)
Dr. Utpala Parthasarathy	Chief Technical Officer (T9)
Mr. K. Jayarajan	Sr. Technical Officer (Stat.) (T6)
Dr. C.K. Sushama Devi	Sr. Technical Officer (T6) (Lib.)

Ms. N. Prasannakumari	Sr. Technical Officer (T6) (Hindi Translator)
Mr. K.T. Muhammed	Technical Officer (T5) (Farm)
Mr. V. Sivaraman	Technical Officer (T5) (Farm) (till 30.11. 2013)
Mr. A. Sudhakaran	Technical Officer (T5) (Artist-cum-Photographer)
Mr. N.A. Madhavan	Technical Officer (T5)

Administrative

Mr.V.L. Jacob	Finance & Accounts Officer (till 30.06.2013)
Mr. V. Mohanan	Administrative. Officer (till 29.07.2013)
Mr. K V Pillai	Administrative Officer (from 02.08.2013)
Mr. M Radhakrishnan	Finance & Accounts Officer (from 09.12.2013)
Mr. K.G. Jegadeesan	Asst. Fin. & Accts. Officer
Mr. C.Venugopalan	Asst. Admn. Officer
Mr. R.N. Subramanian	Asst. Admn. Officer
Ms. P.V. Sali	Private Secretary

IISR Experimental Farm, Peruvannamuzhi

Technical Officers

Mr. V.K. Aboobacker Koya	Chief Technical Officer (T9)
Mrs. E. Radha	Asst. Chief Technical Officer (T(7-8)
Mr. K. Kumaran	Technical Officer (T5)

Krishi Vigyan Kendra

Technical Officers

Mr. P.S. Manoj	Subject Matter Specialist (T9) (Hort.)
Dr. S. Shanmugavel	Subject Matter Specialist (T9) (Veterinary Science)
Mr. K.M. Prakash	Subject Matter Specialist (T9) (Agronomy)
Dr. B. Pradeep	Subject Matter Specialist T6 (Fisheries)
Ms. A. Deepthi	Subject Matter Specialist T6 (Home Science)
Mrs. K K Aiswariya	Subject Matter Specialist T6 (Plant Protection)

IISR Cardamom Research Centre, Appangala

Scientific

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

Administrative

Mr. P. Muraleedharan	Asst. Administrative Officer
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WEATHER DATA 2013

Cardamom Research Centre, Appangala

Months	Temperature (°C)		Rainfall (mm)	Rainy days
	Maximum	Minimum		
January	31.7	12.3	0	0
February	30.8	13.9	48.3	3
March	31.1	17.0	103.6	3
April	33.2	18.4	15.6	3
May	30.0	20.1	57.5	7
June	24.0	16.0	740.3	28
July	23.5	17.4	1494.5	31
August	24.5	17.9	659.3	30
September	28.1	17.3	367.2	25
October	31.9	17.8	168.0	14
November	30.9	16.2	18.3	2
December	31.3	12.1	4.8	1
Average/total	29.25	16.37	3677.4	147

IISR Experimental Farm, Peruvannamuzhi

Month	Temperature (°C)		Rainfall (mm)	Rainy days
	Maximum	Minimum		
January	34.1	19.2	0	0
February	34.8	20.7	20.2	2
March	34.2	22.0	176.4	7
April	35.3	23.4	56.7	3
May	34.3	24.6	376.5	8
June	26.7	21.1	1485.4	27
July	28.3	21.5	1513.2	30
August	28.6	21.6	777.1	24
September	29.9	23.9	422.4	15
October	30.2	24.2	350.8	17
November	32.5	23.3	120.6	6
December	33.1	21.2	17.2	1
Average/total	31.83	22.23	5316.5	140

NEW FACILITIES

- ◆ Infrastructure facilities for high-health planting material production of spices were created with about 640 m² 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.





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