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2011/12

IISR Annual Report

2011/12



भारतीय मसाला फसल  
अनुसंधान संस्थान  
कोषिककोड



Indian Institute of  
Spices Research  
Kozhikode



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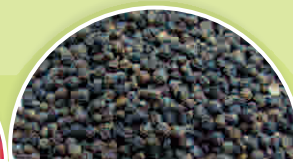
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## PREFACE


I have great pleasure in presenting this Annual Report 2011/12. The institute enriched and maintained its germplasm on all the mandate crops with additions from centers of AICRP on Spices. Endangered species like *Piper barberi* and *P. hapnium* were located from Sabari hills and a variant of *P. nigrum* with lemonish flavor was collected. Single nucleotide polymorphism (SNPs) in the defense related genes viz., osmotin and 1,3 glucanase were identified in *P. colubrinum*. Expression data for specific genes in the *P. colubrinum* and *P. nigrum* transcriptome was developed. An isolate of *Phytophthora* (Is. No. 98-93) infecting black pepper was completely sequenced using next generation sequencing platform and efforts are under way to annotate.

Promising nematode resistant accessions were identified in ginger and turmeric for multi location trials (MLTs). The target yield equations for soil test based fertilizer recommendation for a fixed yield target of black pepper, ginger and turmeric were developed and validated. Based on the research efforts many technologies like micro nutrients requirement for turmeric, nutrient POWER MIX for spices, foliar nutrient sprays and summer irrigation for black pepper, PGPR BIOMIX for ginger and black pepper were identified for transfer to farmers through extension agencies. Seedling progenies of turmeric with more than 4% curcumin content were identified. Protocols for real-time PCR based detection of CdMV and BBrMV in cardamom were standardized. A new species of entomopathogenic nematodes (EPN) belonging to the genus *Osccheius* was identified. The combination product of carbendazim + mancozeb was found to be promising in black pepper and hexaconazole was promising in cardamom for managing anthracnose and leaf blight diseases.

Kisan Mela and Technology Week were organized at IISR Chelavoor during second week of February 2012. The institute participated in three state level exhibitions, fares and one district level exhibition, organized media visits and popularized the technologies through video films, AIR programmes and print media. About 7000 soil samples from farmer's plots have been analyzed for all the essential nutrients and soil health cards with nutrient advisories were given to the farmers. The KVK and ATIC conducted programmes to the farmers' need and trained more than 7000 beneficiaries. Participatory seed production on high yielding varieties of ginger and turmeric was taken up in farmers plots. The KVK made great impact among farmers by providing training on mechanized coconut palm climbing in collaboration with Coconut Development Board.

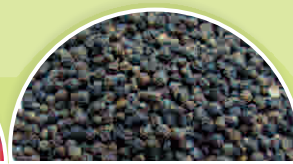
I consider it a privilege to place on record the encouragement and support given by Dr. S. Ayyappan, Director General, ICAR. But for the strong encouragement and guidance we received from Dr. H.P. Singh, Deputy Director General (Horticulture) we would not have made such achievements. We are also grateful to Dr. Umesh Srivastava, ADG (Hort. II) for all the support given to us. I am equally thankful to the Chairman and members of Research Advisory Committee for their suggestions to reorient our research programmes. I appreciate the efforts taken by the staff of this Institute for their support in executing our programmes. I commend the editors for having compiled and brought out this compilation.

Kozhikode  
31 May 2012



**M Anandaraj**  
Director





## EXECUTIVE SUMMARY

### BLACK PEPPER

#### Genetic resources

The present status black pepper germplasm maintained in the nursery and field genebank is 2936 accessions (Wild pepper- 1418, Cultivars-1509, Exotic species- 9). Two hundred and thirty accessions of wild germplasm are conserved at the field genebank of CRC, Appangala. Surveys were carried out in Idukki WLS, Sabari hills and Goodrickal forest range and also in the forests of Kasaragod district. A total of 236 accessions were collected. Endangered species viz., *Piper barberi* and *P. hapnium* were collected from Sabari hills. This is the first report of locating *P. barberi* from Sabari hills. A variant of *P. nigrum* with lemonish flavor was also collected from this area. Besides, two accessions from Assam were collected and added to the germplasm. One hundred and thirty accessions were characterized for eight morphological characters.

#### Screening of mapping population for *Phytophthora* resistance

Fifty seven lines selected as association mapping population were screened using leaf and stem inoculation methods. Two genotypes, Acc. 1324 (Aimpiriyan) and HP 780 (Panniyur 1 × Karimunda) gave most tolerant reaction in three rounds of screening. This hybrid was also found to be resistant to *Phytophthora* in earlier screening trials.

#### Host-pathogen interaction

A genome-wide approach by transcriptomics was undertaken to study *Piper- Phytophthora* interactions with special emphasis on identification of stress induced genes. *P. colubrinum* and *P. nigrum* transcripts showed maximum hit with *Vitis vinifera* (wine grape) sequences, followed by *Populus trichocarpa* (Poplar) sequences indicating closer relationship of magnoliids (order to which *Piper* belong to) with eudicots. The two full length genes (osmotin and  $\beta$ -1,3 glucanase) discovered through transcriptome data analysis was found to have coding sequences corresponding to 913 and 303 amino acids respectively. Comparative gene

expression analysis indicated very high expression of osmotin and  $\beta$ -1,3 glucanase in *P. colubrinum* compared to *P. nigrum*. In the qPCR analysis done on *P. colubrinum*, the expression of  $\beta$ -1,3 glucanase was found to be at its peak at 48 hour post inoculation (hpi) with *Phytophthora capsici* whereas the expression of osmotin was at its peak at 24 hpi. Nine resistance gene related transcripts (NBS-LRR class of R proteins) from *P. colubrinum* were also identified and was found to be related to sequences from *Arabidopsis thaliana*, *Populus trichocarpa*, *Brassica napus*, *Glycine max* and *Hordeum vulgare*. About 15 transcripts from *P. nigrum* were found to be related to plant disease resistance genes.

#### Genetic fidelity testing of tissue cultured (TC) samples

Ten TC samples (five cultures and five hardened plants) were received for genetic uniformity testing from TERI, New Delhi. The samples tested with five ISSR primers short listed for black pepper were found to be genetically uniform.

#### Grafting studies

Grafting of Sreekara on *Piper hamiltoni*, a resistant species gave 50% success but the growth was poor due to susceptibility of the root stock to nematodes. *P. ornatum* resistant to the major pathogens of black pepper was grafted with five promising *Piper sp.* as interstock since the rootstock is not compatible with black pepper. The best combination was *P. hamiltoni* as scion with 100% success. Sreekara gave 80% sprouting with normal growth on *P. ornatum* with *P. hamiltoni* as interstock and this combination appeared promising. However, *P. ornatum* was found to be susceptible to *Sclerotium rolfsii*.

#### Allelopathic effect of tree standards

In the green house study on the allelopathic effect of tree standards on growth of black pepper, the results were in general inconsistent, though the plant height decreased at higher concentrations of 50 and 100%, irrespective of the tree species. The plant height was greatest at 12.5% and 25% concentration but decreased at 50% and 100% concentrations. Results on number of leaves, root



length and fresh weight also followed an identical trend. However, the results did not reveal any allelopathic effects of tree species on growth of black pepper during the period of study.

### Economic optimum for nutrient response

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 ₹ 1.60/standard for N, ₹ 2.40/standard for P and ₹ 5.40/standard for K.

### Molecular profiling of *Phytophthora* isolates

Thirty six SSR primers designed from the EST database of *Phytophthora capsici* were screened for their polymorphism among *Phytophthora* isolates. Twenty nine of the 36 primers amplified the expected amplicon. A total of 35 alleles were scored. The dendrogram showed that the similarity coefficient ranged from 79-100%. Cluster analysis using the un-weighted pair-group method with arithmetic averages (UPGMA) divided the isolates into two major clusters. Also, molecular diversity of 82 *Phytophthora* isolates was studied by sequencing the Internal Transcriber Spacer (ITS) region. The ribosomal DNA region containing the ITS region ITS 1 and 2 and the 5.8S rRNA gene were amplified with the universal primers ITS 6 and ITS 4. All the isolates amplified the expected amplicon of 900bp. BLAST searches for ITS rDNA from these sequences indicated that most of the isolates showed sequence similarity either with *P. capsici* or *P. tropicalis* or both. Two isolates showed sequence similarity with *P. citrophthora*, three with *P. nicotianae*, one with *P. palmivora* and another isolate with *P. infestans*.

### Genomics of *Phytophthora*

A native isolate of *P. capsici* (Is. No. 98-93) infecting black pepper was fully sequenced using next generation sequencing platform, Illumina - Solexa GA II. The sequence data was assembled by taking Joint Genome Institute's *P. capsici* as reference genome.

*In silico* studies on protein-protein interaction in *Phytophthora*-black pepper interaction was conducted. Computational analysis of signal peptide dependent effector proteins in the plant

pathogen *P. capsici* has been carried out. Functional annotation of SNPs in *P. capsici* was carried out and deleterious SNPs were identified through *in silico* analysis.

### Management

Chemicals such as Fenamidone 10%+ Mz 50% (Sectin), Famoxadone 16.6%+ Cymoxanil 22.1% (Equation Pro), Cymoxanil 8%+Mz 64%(Curzate M8), Dimethomorph 50% (Acrobat 50) reported as effective against *Phytophthora* species in other crops were evaluated both *in vitro* and *in planta* against *P. capsici* in comparison with existing recommendation of Metalaxyl- Mz. Among the fungicides, Fenamidone- Mz (450 ppm.) was found to be the best in preventing foliar infection and root infection and is comparable to Metalaxyl- Mz at the recommended dosage (1250 ppm). No lesion development was observed even when inoculated after 14 days of fungicide imposition.

Twelve phytochemicals from phenylpropanoid pathway were docked to modeled  $\beta$ -1,4 endoglucanases (EGase) enzyme of *Radopholus similis* to assess their binding affinity and consequently their inhibitory activity. Based on binding energy scores such as moldock score and re-ranking, maximum inhibitory activity was found in syringin followed by sinapaldehyde and sinapic acid. Ferulic acid @ 250 and 500 ppm reduced *R. similis* population in rooted cuttings.

Profiling and activity prediction of biochemical compounds using *in silico* tools were completed for *Pseudomonas putida* BP 25 and *Bacillus megaterium* BP 17. Potential antioomycete secondary metabolites were identified from *P. putida* through virtual screening and *in silico* docking studies. Similarly, around 35 potential metabolites having nematicidal activity were identified from *B. megaterium*.

Field evaluation of three promising disease/nematode resistant lines *viz.*, Hp 39, IISR Sakthi, C 1090 and Sreekara with biocontrol agents such as *T. harzianum*, *P. fluorescens* (IISR 6), *P. aeruginosa* (IISR 853) and *Pochonia chlamydosporia* showed that IISR Thevam and Sreekara are highly responsive to *T. harzianum* with 100% establishment followed by IISR 6.





### Development of Real-Time PCR for detection of viruses

SYBR green based real-time PCR was developed for detection of *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV). The protocol involved total DNA/RNA isolation and subjecting them to real time PCR using specific primers for each of the viruses. This method was ten times more sensitive than conventional PCR.

Studies to know the location of the virus in seeds indicated the presence of PYMoV in all seed parts such as embryo, endosperm and perisperm in all the three varieties tested and the concentration of viral DNA was almost the same in all the parts.

### Anthracnose incidence in relation to weather

Observations on anthracnose incidence and weather variables *viz.*, maximum and minimum temperature, number of rainy days and rainfall showed that though the disease was prevalent at lower levels during February (8.3%), March (7.5%), April (7.5%) and May (14.7%), a rapid increase in the disease incidence was noticed in June (22.1%) and subsequently registered a peak in September (39.1%). Among the temperature variables,  $T_{max}$  had a negative correlation and  $T_{min}$  had a positive correlation with disease progression. Disease incidence was also maximum during the months which had more number of rainy days and it was inferred that both rainfall and number of rainy days have positive correlation with disease progression.

### Survival of pathogen

Survival of *Colletotrichum gloeosporioides* in the infected plant part (leaves) was studied under laboratory, greenhouse and field conditions. The fungus survived in the infected plant debris for about three months under field conditions and under laboratory conditions for more than four months. A simple method was also devised for the production of microsclerotia under *in vitro* conditions and for their easy separation into single units. Formation of microsclerotia was observed 7–8 days after the incubation period. Three types of microsclerotial germinations were observed under laboratory conditions *viz.*, sporogenic (production of conidial mass),

myceliogenic (production of hyphae) and both sporogenic and myceliogenic germinations.

### Management

The efficacy of different fungicides was evaluated against anthracnose in the nursery. The newly emerged leaves in cuttings treated with Carbendazim + Mz (0.1%) were free from the disease when compared with other fungicides. The treatment also delayed disease development by 15 days.

### Pollu Beetle - biochemical characterization

The total essential oils and phenols and surface wax were estimated in berries of three pollu beetle resistant black pepper accessions (816, 841 and 1114) and one susceptible variety (Panniyur 1). The total essential oil in immature berries of susceptible accession (Panniyur 1) was 3.98% and in resistant accessions it ranged from 2.8% to 8.6%. The total phenol content in susceptible accession was 0.613  $\mu\text{g/g}$  and varied from 0.735 to 1.011  $\mu\text{g/g}$  in resistant accessions. The surface wax in the resistant accessions of immature berries varied from 0.4782 to 0.6917  $\mu\text{g/g}$  and in susceptible accession it was 0.2926  $\mu\text{g/g}$ .

## CARDAMOM

### Germplasm characterization

Cardamom field gene bank was enriched with 17 accessions (from RRS, Mudigere) bringing the total to 562. Morphological characterization was done in 50 accessions raised in the field gene bank. The accessions *viz.*, IC 547146 (yield), IC 547161 (yield), IC 547147 (oil & yield), IC 349646 (leaf blight resistance) and IC 547223 (rhizome rot resistance) were shortlisted for future comparative yield trials.

### Evaluation of hybrids

Analysis of four successive crop yields of  $F_1$  hybrid progenies of Preliminary Evaluation Trial I & II resulted in identifying the following hybrids: NKE-12  $\times$  MB-5 (1499 kg/ha), MB-5  $\times$  NKE -19 (1461 kg/ha), GG  $\times$  NKE-12 (1350 kg/ha), RR-1p  $\times$  CCS-1 (1245/868 kg/ha), CCS-1  $\times$  RR-1 (1022/765 kg/ha), ASH (1930/1119 kg/ha), NKE-12  $\times$  GG (1746/741 kg/ha), GG  $\times$  NKE -19 (1635/833 kg/ha).



### Performance of elite lines under moisture stress

Twelve short listed genotypes with three checks were studied for growth and yield parameters under stress. IC 584058 (APG 474) recorded early yield, good setting and bold capsules. It recorded 32 total tillers per clump, 44 panicles per clump, 176.8 capsules per panicle and 3889.6 capsules per clump and more than 80% capsule were bold (>8mm). IC 584078 (GG×893), a multibranching panicle genotype recorded 85 panicle per clump, 71.2 capsules per panicle, 6072 capsules per clump with more than 50% bold green coloured capsule (>8mm), and took longer time to leaf folding when exposed to sunlight.

### Evaluation for quality

Sixty nine accessions of cardamom were evaluated for quality and the essential oil content in these accessions ranged from 2.4 - 5.0%. Highest essential oil content was recorded in GG and Acc. 547184. GG contained 20.8% 1,8-cineol and 48.1%  $\alpha$ -terpinyl acetate whereas Acc. 547184 contained 24.1% 1,8-cineol and 49.8%  $\alpha$ -terpinyl acetate. These two accessions showed similar composition except that GG contained relatively higher levels of limonene and geraniol compared to Acc. 547184.

### Molecular characterization of small cardamom

Molecular profiles were developed for 100 accessions using 25 ISSR markers for studying the genetic diversity. The study clearly indicated the diversity among small and large cardamom accessions selected for developing core collections. Ten TC samples of large cardamom were received for genetic uniformity testing from TERI, New Delhi. The samples were found to have partial instability in three lines with one of the five IISR primers tested

Around 270 clones from cardamom genomic DNA were isolated and sequenced using hybridization – enrichment method. Twenty four primers were designed and 20 primers gave good amplification in small cardamom.

### Development of Real-Time PCR for detection of viruses

A protocol for SYBR green based real-time RT-PCR for detection of *Cardamom mosaic virus*

(CdMV) and *Banana bract mosaic virus* (BBrMV) was developed. The protocol involved total RNA isolation and subjecting them to real time PCR using specific primers for each of the viruses. Seed transmission studies were carried out using immature capsules, immature and mature seeds collected from cv. Njallani Green Gold. In RT-PCR, all the samples showed a clear amplification of 950 bp for BBrMV and 1050 bp for CdMV, indicating the presence of the viruses in the plant parts tested. However, all the seedlings were asymptomatic and the absence of both the viruses in the seedlings was confirmed by RT-PCR.

### Rhizome rot/Root rot

Surveys conducted in Wayanad and Idukki districts of Kerala, Hassan and Kodagu districts of Karnataka to study the seasonal variation of rhizome and root rot diseases showed high incidence and severity in Meppadi region of Kerala and Appangala and Kadagudalu regions of Karnataka. Eighty five isolates of fungi were isolated from the diseased samples, which included *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium* spp, *Colletotrichum* sp, *Pythium vexans*, *Botryodiplodia theobromae* and unidentified cultures. Pathogenicity of 10 *Fusarium* isolates was tested on IISR Kodagu Suvasini and Appangala isolate caused complete wilting of the seedling within six days after inoculation. Morphological characterization of 10 *Fusarium* and eight *Rhizoctonia* isolates was completed.

### Lead Spot

The survival of *C. gloeosporioides* infecting cardamom in infected plant part (leaves) was studied under laboratory, greenhouse and field conditions. The fungus survived in the infected plant debris for about three months under field conditions. Under laboratory conditions the fungus survived for more than four months.

### Thrips

Out of three hundred and sixty accessions screened for the damage caused by cardamom thrips on capsules in the field, all the accessions screened were susceptible and the percentage of infested capsules ranged from 5%-100% in various accessions. Eighteen accessions had



thrips damage below 10% with the lowest damage on IC-379596 (5%). Capsule damage was more than 90% on 22 accessions. Accessions IC-349457, IC-349350 and GG recorded 100% capsule damage. Collections of cardamom thrips were made from Vythiri and Kurupalakotta (Wayanad District, Kerala) and adults and larvae were surface sterilized and the endosymbionts associated with them were studied. Ten isolates were identified through biochemical methods and Biolog. *Enterobacter cloacae* and *Bacillus subtilis* were the common bacteria isolated from the adults. The larvae also yielded *Bacillus pumilus*.

Eleven insecticides and natural products which included new molecules were evaluated against thrips in the field and all treatments except dinotefuron were significantly superior in reducing the damage caused by thrips when compared with control. The lowest damage was observed in plants treated with Fipronil (0.6%) which was on par with Thiomethoxam (1.3%), Imidacloprid (1.9%) Spinosad (2.1%), Zolone (3.4%) and Thiocloprid (3.4%).

## TURMERIC

### Genetic resources

Wild germplasm exploration and collection were carried out in the Thekkady forests, Idukki district, Kerala. *Curcuma longa* and *C. aromatica* were collected and conserved in the *ex situ* gene bank at IISR, Peruvannamuzhi. The turmeric, most probably a run wild entity, is characterized by moderate yield (fresh clump weight-1.23kg), dry recovery-17.19%, moisture-8.19%, oil-3.2%, curcumin- 5.42% and oleoresin-13.78%.

### Promising lines

The shortlisted nematode tolerant accessions of turmeric (7) were evaluated for morphological and yield characters and Acc. 79 and Acc. 48 were found to be promising. Thirteen seedling progenies showed curcumin content above 4%. Seedling progeny 389/1 showed above 5% curcumin consistently for three years.

Chromosome number was analyzed in 60 seedling progenies. All of them showed deviation from normal chromosome number of  $2n=63$ . Most frequently occurring number was  $2n=84$ . Of the two mother plants analyzed, one showed  $2n=63$  and other  $2n=84$ .

### Economic optimum for nutrient response

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 ₹ 0.65/ bed for N, ₹ 0.40/ bed for P and ₹ 0.85/bed for K.

### Organic farming

In turmeric, the rhizome yield was higher under integrated system followed by organic and chemical system. Significantly higher oleoresin and curcumin contents were recorded under organic on par with integrated management. Under organic management, Alleppey Supreme recorded higher oil and starch (50%) contents whereas Prathibha recorded higher curcumin (5.6%).

### Micronutrients on yield and quality

The effect of Zn and B on the quality of turmeric var. Prathiba was studied. The pooled analysis of three years yield data showed an increased response with soil application of Zn up to 10 kg/ha which tended to decrease at a higher dose of 15 kg/ha. Application of one or two foliar sprays of  $ZnSO_4$  (0.25%) also recorded yield on par with that of soil Zn application. Foliar spray of Zn twice @ 0.25% and soil application @ 10 kg/ha recorded higher curcumin (4.9-6.2 %) and oleoresin (11.2 – 13.5%) contents. Similarly, application of B @ 1 kg/ha without lime application increased the rhizome yield up to 15% compared to control. Foliar spray of B once or twice @ 0.2% recorded significantly highest yield of 14.8 and 13.7 kg/ 3m<sup>2</sup> respectively and also increased the curcumin content significantly (4.86 – 6.08%).

### Cloning of pal gene

PCR conditions have been optimized using pal gene specific primers, designed based on plant transcript assembly database at TIGR. PCR amplified products of 1336, 1335 bp length were obtained using templates of (var. Alleppey Supreme) rhizome DNA. These were cloned into PTZ57R/T and sequenced. Blast analysis revealed sequence identity of up to 97% with pal sequences of *Zea mays*, *Musa balbisiana*, *Oryza*





*sativa* Japonica Group and *Salvia miltiorrhiza*.

### Essential oil profile

The chief components of the essential oil of *C. longa* were turmerone (30.6%), ar-turmerone (5.08%) and curlone (15.03%); myrcene (37.2%), and  $\beta$ -pinene (9.03%) in *C. amada*; curdione (13.38%), camphor (9.38%), 1,8- cineole (6.81%), borneol (4.85%), germacrone (3.93%), camphene (3.07%),  $\beta$ -elemene (3.3%) curzerene (4%) and neocurdione (4%) in *C. aromatica*; curzerenone (17.91%), 1,8- cineole (9.26%), camphor (2.9%), bornyl acetate (3.6%),  $\alpha$ -terpineol (2.56%), curzerene (5.2%), and  $\beta$ -elemene (4.64%) in *C. caesia*. Essential oil content in 13 varieties of turmeric viz., Sugandham, Roma, Suroma, Pant Pithab, Renga, Co-1, BSR-1, Rajendra Sonia, Varna, Suranjana, Resmi and Sona ranged from 3.1-5.7%. Highest essential oil content was observed in Resmi (5.7%) followed by Suroma and Renga with 5% each. The major constituents of the essential oil were  $\alpha$ - phellandrene (0.5-5.9%), 1,8- cineol (0.2-2.2%), terpinolene (0.4-5.1%), ar-curcumene (0.9-4.1%), zingiberene (1.4-10.3%),  $\beta$ -sesquiphellandrene (0.7-10.4%), turmerone (22.6-45.1%) and curlone (6.8- 21.7%). Turmerone content was maximum in the cultivar Sugandham (45.1%), followed by Roma (40.9%). Resmi contained 31.9% turmerone and 21.7% curlone.

## GINGER

### Genetic resources

Wild germplasm exploration and collection were carried out in the Thekkady forests, Idukki district, Kerala. A putative wild type (*Zingiber officinale* (Wild)) was collected and conserved in the *ex-situ* gene bank at IISR, Peruvannamuzhi. The *Zingiber officinale* is characterized by very small rhizomes, reduced tillers, medium stature and less abundance.

Pooled analysis of the yield and quality data of Nepal ginger accessions indicated the superiority of Accs. 578, 581 and 593. The shortlisted nematode tolerant accessions of ginger were evaluated for morphological and yield characters and Acc. 219 was found to be promising with high yield.

Among the 116 M<sub>6</sub>V<sub>6</sub> and 181 M<sub>5</sub>V<sub>5</sub> generation plants of irradiated varieties screened against

*Ralstonia solanacearum*, three mutants were found to survive the infection even after third round of screening.

### Evaluation of PGPR strains for biocontrol

Ginger rhizome treated with 1% starch solution containing bacterial suspensions ( $\sim x 10^{10}$  cfu mL<sup>-1</sup>) of *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB 68) recorded higher sprouting, plant growth promotion and lesser disease incidence.

Promising native strains of PGPR [GRB- 25 (*Burkholderia cepacia*), GRB 36-(*Klebsiella* sp.), GRB 38- (*Serratia marcescens*) and GRB 70- (*Enterobacter* sp)] when applied alone or in combination with varying rates of NPK fertilizers positively influenced microbial biomass-C, -N, -P, soil respiration, and enzyme activities.

### Economic optimum for nutrient response

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 ₹ 3.75/ bed for N, ₹ 1.30/ bed for P and ₹ 0.60/bed for K.

### Organic farming

Integrated management recorded significantly higher oil and varieties Varada, Rejatha, Mahima were on par under different management systems. Oleoresin content was significantly higher under organic management in Varada (4.5%) on par with chemical management.

### Source- sink relationship and endogenous hormone levels in ginger

Partitioning studies in three different varieties viz., IISR Varada, IISR Rejatha and IISR Mahima showed that at 50 days after planting (DAP), shoots received 70-74 % biomass and rhizomes received 18-21% of the total biomass. At 150 DAP, shoots received 23-33 % and rhizomes received 63-74 % of the total biomass. At 50 DAP, rhizomes had only 5 - 6 % starch while at 150 DAP rhizomes accumulated 49 - 56.2 % starch. Among the three varieties, IISR Varada showed highest photosynthetic rate (9  $\mu$  moles m<sup>-2</sup> s<sup>-1</sup>) and biomass accumulation in rhizomes (74 % at 150 DAP). Accumulation of auxin (44-66 pico





moles) and cytokinin (18.5–31.2 pico moles) was maximum during rapid rhizome development (150 DAP).

### Bacterial wilt

MLST analysis of five housekeeping genes, dispersed in the chromosome, and three virulence-related genes, located on the megaplasmid was done to classify *Ralstonia solanacearum*. PCR amplification of housekeeping genes (*ppsA*, *adk*, *gapA*, *gdhA*, *gyrB*) and virulence genes (*hrpB*, *fliC* and *egl*) in 21 strains of *R. solanacearum* was compared. Allele numbers were obtained by sequence comparison with alleles documented in the database [www.pamdb.org](http://www.pamdb.org). Several novel alleles could be found in ginger strain of *R. solanacearum* showing the diversity within the biovar *R. solanacearum*. rec N, a gene coding for DNA repair protein, was used for phylogenetic analysis of *R. solanacearum* representing different hosts and geographical locations in India and it was observed that rec N can be used as a tool for classifying *R. solanacearum* into different phylogenetic groups.

Actinomycetes isolated from the rhizosphere of healthy plants were evaluated against *R. solanacearum* *in vitro* and *in planta*. Among the 24 isolates, nine isolates showed *in vitro* inhibition and one isolate (Act 4) was promising in *in planta* inhibition of the pathogen by reducing the disease incidence to 79% when compared to control. The isolates were characterized morphologically and by molecular methods by *rpoB* (RNA polymerase  $\beta$  subunit) gene sequencing. Sequence information showed that all the potential isolates belonged to *Streptomyces* species. One isolate was identified as *Kitasatospora setae*. Field experiments conducted at Peruvannamuzhi indicated that *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB68) were effective for disease control and plant growth promotion.

### Shoot Borer

Leaf cuticle wax and total phenols were estimated in mature leaves and shoots of seven moderately resistant (Accs. 171, 203, 227, 247, 252, 260, and 432) and five susceptible (Accs. 137, 191, 208, 480 and 495) accessions. The wax content in susceptible and moderately resistant accessions

ranged from 0.0050 to 0.0067 and 0.0054 to 0.2800 mg/200 cm<sup>2</sup> respectively. The total leaf phenol content in susceptible and moderately resistant accessions varied from 2.08 to 3.20 and 2.88 to 4.46 mg/g of dried leaf, respectively. Leaf cuticle wax was estimated in six moderately resistant (Accs. 422, 435, 589, 687, 954 and 1026) and three susceptible (Accs. 924, 925 and 1007) accessions. The wax content in susceptible and moderately resistant accessions ranged from 0.0058 to 0.0086 and 0.0055 to 0.3311 mg/200 cm<sup>2</sup> respectively.

Multiplication of infective juveniles of eight promising isolates of EPNs was studied on five artificial media *viz.*, Wouts media, egg yolk media, dog biscuit media, agar agar media and wheat flour media. Maximum number of infective juveniles of EPN-IISR 04 and EPN-IISR 06 were obtained in egg yolk and Wouts media, respectively. Multiplication of EPNs on wheat flour medium was very low. No multiplication was observed in agar agar and dog biscuit media.

Two isolates of EPNs *viz.*, *Steinernema abbasi* (NBAIL SA 01) and *Heterorhabditis indica* (NBAIL Hi 1) obtained from NBAIL, Bangalore, were tested for their infectivity on larvae and pupae of shoot borer under laboratory conditions. Both the species were highly pathogenic to shoot borer larvae resulting in 100% mortality within 48 h. However, only 17% and 33% mortality was caused by these isolates against shoot borer pupa. One species of EPN collected earlier belonged to the *Oscheius gingeri* and was identified as new species on the basis of morphological and molecular characterization.

## TREE SPICES

### Genetic resources

Seedlings of a unique nutmeg type with more of hermaphrodite flowers have been collected from Sr Poornanand V Bhat, an innovative farmer from Aversa, Ankola (Uttar Kannada).

### Garcinia butter

Hot water extraction and solvent extraction (Methanol/chloroform -1:1) of *G. gummigutta* and *G. tinctoria* seeds yielded 50% butter with yellow colour and pleasant aroma. The butter had saponification value of 189.9, 1.42% free fatty acid and sterol (0.5%).



## VANILLA

### Screening for disease resistance

Ten plants each derived from irradiated protocorms with 0.5 kr and 10kr gamma rays and interspecific hybrids involving *V. planifolia* × *V. tahitensis* were inoculated with *Fusarium oxysporum*. After three rounds of inoculation one plant derived from the protocorms treated with 0.5kr was found to be free of infection. Others showed varied levels of infection and plant death. Later, mild infection was noticed in plant initially found free of infection.

Chromosome number analysis of two interspecific hybrids between *V. planifolia* and *V. tahitensis* showed  $2n=30$  in one (PT-5) and  $2n=32$  in the other (PT-17).

## PROCESSING & VALUE ADDITION

### Flavour quality of black pepper

Black pepper sample powdered in pin mill was exposed to 50°C and stored in open and packed condition for ten days and evaluated for oil, oleoresin, piperine, total phenol and essential oil constituents. Essential oil showed a reduction of 22% in packed and 50% in open condition. Oleoresin showed 13% reduction in packed and open condition and piperine content did not show any significant change. Low boiling pepper specific flavor constituents like pinene, sabinene, myrcene and limonene were affected. High boiling constituents such as caryophyllene did not show any change. The anti oxidant property as analysed by DPPH radical scavenging assay, phosphomolybdenum assay and ferric reducing power did not show any change.

### Flavour quality of turmeric

Turmeric var. Prathiba sample powdered in pin mill was exposed to 40°C and stored for ten days and evaluated for oil, oleoresin, curcumin, total phenol and essential oil. Reduction was found only in essential oil (about 15%).

### Curing techniques in turmeric

Studies on curing of turmeric (variety Prathiba) were conducted in TNAU model steam boiler and by water boiling method. The results indicated that slicing significantly reduced the drying time (8 days). Turmeric cured in improved boiler for 30, 45, 60, 90 min took 18, 16, 11 and 10 days for drying whereas traditional water boiling for 40,

60, 90 min took 10 days for drying. The reduction in curcumin, starch, essential oil, oleoresin and drying time with increased curing time was highly significant by both methods.

### Nutraceutical properties of bioactive compounds in spices

Four cancer cell lines, HeLa (human cervical carcinoma cells), MDA-MB-231 (human breast carcinoma cells), HepG2 (human hepatocellular carcinoma cells) and A375 (human melanoma cell line), were treated with essential oil of black pepper, ginger, turmeric, cinnamon, and curry leaves, water and ethanol extracts of black pepper, ginger, turmeric, cinnamon, *Garcinia indica*, *G. gummi-gutta*, tamarind and curry leaves at two concentrations (at 25 µg/mL and 50 µg/mL). Essential oil of ginger, turmeric, cinnamon and curry leaf showed significant decrease in cell viability. Turmeric, cinnamon and curry leaf reduced the viability of HeLa cells by as much as 15-18%. Water and ethanol extracts were less cytotoxic than the essential oils, the most effective were turmeric, *G. gummi-gutta*, *G. indica*, curry leaf, tamarind and cinnamon, depending on concentration and cell line. The ethanol extracts were superior to water extracts.

### Production of food extrudates

The result obtained from the extrusion process of cassava flour blended with different spice powders indicated that the flour blended with cardamom powder and black pepper powder had good overall acceptability scores of 6.3 and 5.9 respectively. Extrudates of cassava + cardamom and cassava + black pepper had the expansion ratios of 2.84 and 3.17 respectively which were high in comparison with the extrudates from the other blends.

### Management of mycotoxin contamination in spices

Turmeric leaf oil and cinnamoum cassia bark oil were tested for the inhibition of aflatoxin production by *A. flavus* at concentrations ranging from 0.01% to 1.5 % and 0.01% to 0.5% respectively. Complete inhibition was seen at 1.5% (v/v) with a drastic reduction in the aflatoxin content from 163 ppb, at 0.75% of the oil to 4.3 ppb at 1.0%. The optimal protective dosage of 1.5% leaf oil *in vitro* stands good in terms of its



practical utility. Cinnamom cassia bark oil showed complete inhibition of the fungal growth at 0.5%. *In vitro* studies using bacterial antagonists, viz., *Pseudomonas aeruginosa*, *P. putida*, *Bacillus megaterium* and *Curtobacterium luteum* revealed *P. aeruginosa* as the most potent species to prevent the growth of *Aspergillus flavus* in culture. The inhibitory activity was confirmed through the production of extra cellular metabolites in culture which were heat stable.

### BIO INFORMATICS

A new database, *Phytophthora* Genome Database (<http://220.227.138.212/genomedb/>) based on *Phytophthora* whole genome sequencing and annotation was developed. The database provides access to primary structure of the *Phytophthora* genome including genome sequence, number of genes, CDS, SNPs, inDels, nucleotide composition, intron-exon structure, start and stop codon, intron lengths, alternative splicing and untranslated regions (UTRs) to the research community. GenomeView, a next-generation stand-alone genome browser and editor developed at Broad Institute is used as the genome browser. Another database on *Radopholus* genus called RADOBASE (<http://www.spices.res.in/radobase>) was developed and launched. This database contains comprehensive information on sequence and morphological details of 22 *Radopholus* species.

### EXTENSION AND TRAINING

During the year, 897 farmers (365 from within district, 220 from the state and 312 from outside state), 922 students availed farm advisory services from ATIC. Eleven groups of farmers visited under sponsored study tour programmes. Two courses for twenty two trainees, on production management of ginger and turmeric were organized, one for a farmers club from SAS Nagar, Punjab and another for a Seed Cooperative Society from Raipur, Chattisgarh. Exposure training programmes were organized for new field officers and scientists recruits of Spices Board and for a group of field extension officers of Department of Horticulture, Kodagu district, Karnataka. Planting materials worth ₹ 182290/- and

publications worth of ₹ 18770/- were distributed during the year. A 44% increase in sale of *Trichoderma* and *Pseudomonas* formulations worth ₹ 147995/- was recorded, which is 15 times higher over the previous year.

### Soil based plant nutrient management plan

As on date, 5054 soil samples have been analyzed for pH, EC, Organic C, Bray P, Exchangeable-K, -Ca-, -Mg, micronutrients, B and S. Data of 16 Panchayats (3925 nos) and details of 6412 farmers have been uploaded. Mid-term results indicated that all the soils were acidic, mean available P level was  $114.35 \pm 83 \text{ kg ha}^{-1}$  and 9% of the samples had high (25-35  $\text{kg ha}^{-1}$ ), 30% had very high (36-100  $\text{kg ha}^{-1}$ ) and 25% of the soils recorded extremely high ( $> 100 \text{ kg ha}^{-1}$ ) available P. Mean exchangeable Mg level was  $90 \pm 78 \text{ kg ha}^{-1}$  and only 19% of the samples were adequate in exch. Mg ( $> 120 \text{ kg ha}^{-1}$ ) and a major percentage of the samples were inadequate in exch. Mg (37% low and 44% very low). Nutrient advisories have been generated for 22 Panchayats.

### Multi-enterprise farming models to address the agrarian crisis of Wayanad District

During the past year 9000 cuttings of black pepper of varieties of IISR, Calicut were supplied to RARS, Ambalavayal. Varieties supplied are Malabar Excel, Panchami, Sreekara, Girimunda, Thevam, Pournami, Shakthi. Training on IPM and IDM in major spices held at Wayanad Social Services Society, Mananthavady on 26<sup>th</sup> May 2011. FLD on ginger (Varada) successfully conducted at Puthankunnu, S. Batheri. Impact assessment on the 5000 black pepper cuttings distributed at Mananthavady during 2009-2010 was made. The study indicated 83% survival/establishment.

### Technology mission for pepper in Wayanad

About 1500 soil samples from black pepper growing Panchayats of Wayanad district were analysed for major, secondary and micro nutrients and results with site specific recommendations was passed on to the farmers. 15 % of the soil samples analysed were found to be highly acidic, needing immediate application of amendments like lime/ dolomite. In case of available P, 62% of samples analysed were found to have  $> 40 \text{ kg/ha}$





among which >35% of soils had >100 kg/ha P, which is very high. Ten visits were made by team of scientists to disease hot spot/ problem areas along with KAU scientists and advisories were given to the farmers. Pamphlets were prepared in Malayalam on composting, use of pesticides, Biocontrol of pest and diseases and distributed to farmers.

Two farmers per Panchayat are selected and FLDs on varieties and technologies have been initiated. All the inputs like planting material, organic manures, neem cake and bio agents were supplied and is being supervised through field assistants.

### Media visits

Three media visits were arranged to various demonstration units of KVK and IISR experimental farm and progressive farmers fields. Eight journalists from various english/malayalam newspapers/farm magazines and radio channels participated in the visits. More than 40 Success Stories and 130 news items (Coverage of Kisan Mela, Success Stories, Media Visits, New Varieties, Technologies, etc) were published.

### Audio/ video programmes

Five radio programmes and four TV news clippings were produced and 15 audio capsules were broadcasted through AIR, Calicut. Special programmes on IISR and spices cultivation were broadcasted in Mattoli FM and AIR Kannur FM.

Four documentary films were produced on,

- Rewriting the fate of Queen of Spices: A Success Story from Kodagu
- George Panackavayal: Harvester of Hope
- Cassava - The Bread of Tropics: Ensuring livelihood to poor farmers
- *Kalpavriksha* brings happiness in their homes

### Kisan mela and technology showcasing

'Krishi Jalakom 2012' & Technology Expo were organized from February 16-18, 2012. Mr. Sparjan Kumar, IPS, District Police Chief inaugurated the farmers meet. Dr. M. Anandaraj, Director, IISR presided over and Dr. M. Tamil Selven, Director, DASD, Kozhikode opened the exhibition stalls. Twenty organizations including public and private displayed their technologies

and products in the exhibition. Over 400 farmers attended the meet and more than 1000 people visited the stalls. Turmeric farmer Mr. Chandrasekhar Azad, Andhra Pradesh, Dairy Farmer Mr. John Joseph, Kodanchery, Black Pepper farmer Mr. George, Vazhapparambil and Remote operated Coconut climbing machine developer Mr. Prakash, Kozhikode were felicitated on the occasion.

### Awareness programme on PPV&FR Act, 2001

An Awareness programme on the Provisions of "Protection of Plant Varieties and Farmers Rights (PPV&FR) Act 2001" Sponsored by PPV&FRA, New Delhi was organized at institute on Friday, 17 February 2012. Ms. R. Ushamani, Principal Agricultural officer, Kozhikode, inaugurated the programme and Dr. S. Ramachandran, Director, Regional Science Centre and Planetarium Kozhikode, presided over the function. One hundred and forty registered farmers participated actively in the awareness programme. Agricultural Officers and Scientists from different institutions also participated in the function.

## INSTITUTE TECHNOLOGY MANAGEMENT UNIT

The following technologies developed by IISR are ready for commercialization through the unit.

*A simple and easy PGPR technology for ginger:* This PGPR formulation enhances nutrient mobilization and nutrient use efficiency, growth and yield and provides protection against diseases at a negligible cost. It can be applied to rhizomes prior to planting. Booster doses of the same PGPR can be given as soil drench.

*A new microbial consortium for enhanced growth and yield in black pepper:* It can be applied both in black pepper nurseries and under field condition as soil drench or along with FYM. Roots when dipped in microbial formulation improves rooting and performance of plants.

*Nutrient mix for enhanced growth, yield and quality of spices:* This is a novel soil pH based micronutrient mixture for promoting growth, yield and quality of turmeric, ginger & cardamom. Under proper conditions it can be stored for up to one year/ one crop season. It is recommended as foliar spray at the rate of 5 g/litre



on 60<sup>th</sup> and 90<sup>th</sup> day after planting in case of turmeric and ginger and as foliar spray at the rate of 5 g/litre in May-June and September-October every year in case of black pepper and cardamom. An approximate increase of up to 15% in yield and a cost benefit ratio of 1:2.5 are expected.

### KRISHI VIGYAN KENDRA

KVK has conducted 162 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 6324 trainees were benefitted out of the programmes. Ten Front Line Demonstrations and eight On Farm Trials on technology assessment and refinement were carried out during this period. The Kendra made great impact among farmers by providing training on mechanized coconut palm climbing in collaboration with Coconut Development Board, empowering many including women as successful climbers. Through Plant and Animal Clinic, 682 consultancy services, 2572 vaccination of poultry birds and animals and 11 animal health campaigns were conducted. Participatory seed production on high yielding varieties of ginger and turmeric was taken up in 10 farmers plots yielding 400 kg turmeric (IISR

Prabha) and 800 kg of ginger (IISR Varada). The Kendra conducted three seminars and one study tour, participated in five Kisan Mela cum exhibitions, broadcasted nine radio talks for farmers. During this period, ₹ 11.78 lakhs was realized through sale of various technological inputs to farmers.

### HUMAN RESOURCE DEVELOPMENT

- One month summer training on Biochemistry, Biotechnology and Bioinformatics was conducted for 14 M.Sc. students during 3<sup>rd</sup> May – 4<sup>th</sup> June 2011.
- Eight M.Sc./M.Tech students carried out project work in various disciplines.
- Three students were awarded Ph.D.

#### National training programme on 'Allele Mining'

A national training programme on *Allele Mining* sponsored by National Agricultural Innovation Project, was organized at this institute during September 12-25, 2011. The national training, exposed the trainees, the use of genomic technologies along with genetic and bioinformatics approaches for identifying allelic variations and to dissect trait-gene associations



## INTRODUCTION

### History

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

### Location

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

### Mandate

- To extend services and technologies to conserve genetic resources of spices as well as soil, water and air of spices agroecosystems.
- To develop high yielding and high quality spice varieties and sustainable production and protection systems using traditional and non-traditional techniques and novel biotechnological approaches.
- To develop post harvest technologies of spices with emphasis on product development and product diversification for domestic and export purposes.
- To act as a centre for training in research methodology 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.

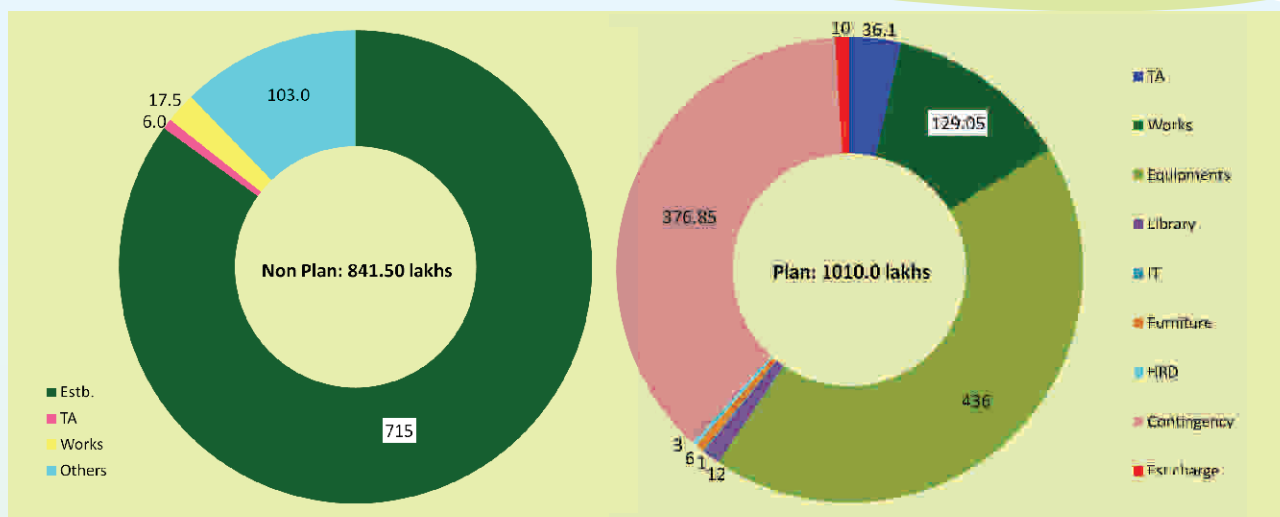
The spice crops on which research is being conducted at the institute include black pepper (*Piper nigrum*), cardamom (*Elettaria cardamomum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), cinnamon (*Cinnamomum verum*), cassia (*C. cassia*), clove (*Syzygium aromaticum*), nutmeg (*Myristica fragrans*), allspice (*Pimenta dioica*), Garcinia (*Garcinia gummi-gutta* and *G. indica*) and vanilla (*Vanilla planifolia*).

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

**Budget:** The total budget of the institute was ₹ 1851.50 lakhs during the year, which included ₹ 1010.00 lakhs (including OPR on *PhytoFuRa*) under Plan and ₹ 841.50 lakhs under Non Plan.





Resource generation: Institute earned a total of ₹ 32.0 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 two administrative, 12 technical and two supporting staff.

### Staff Position of the Institute

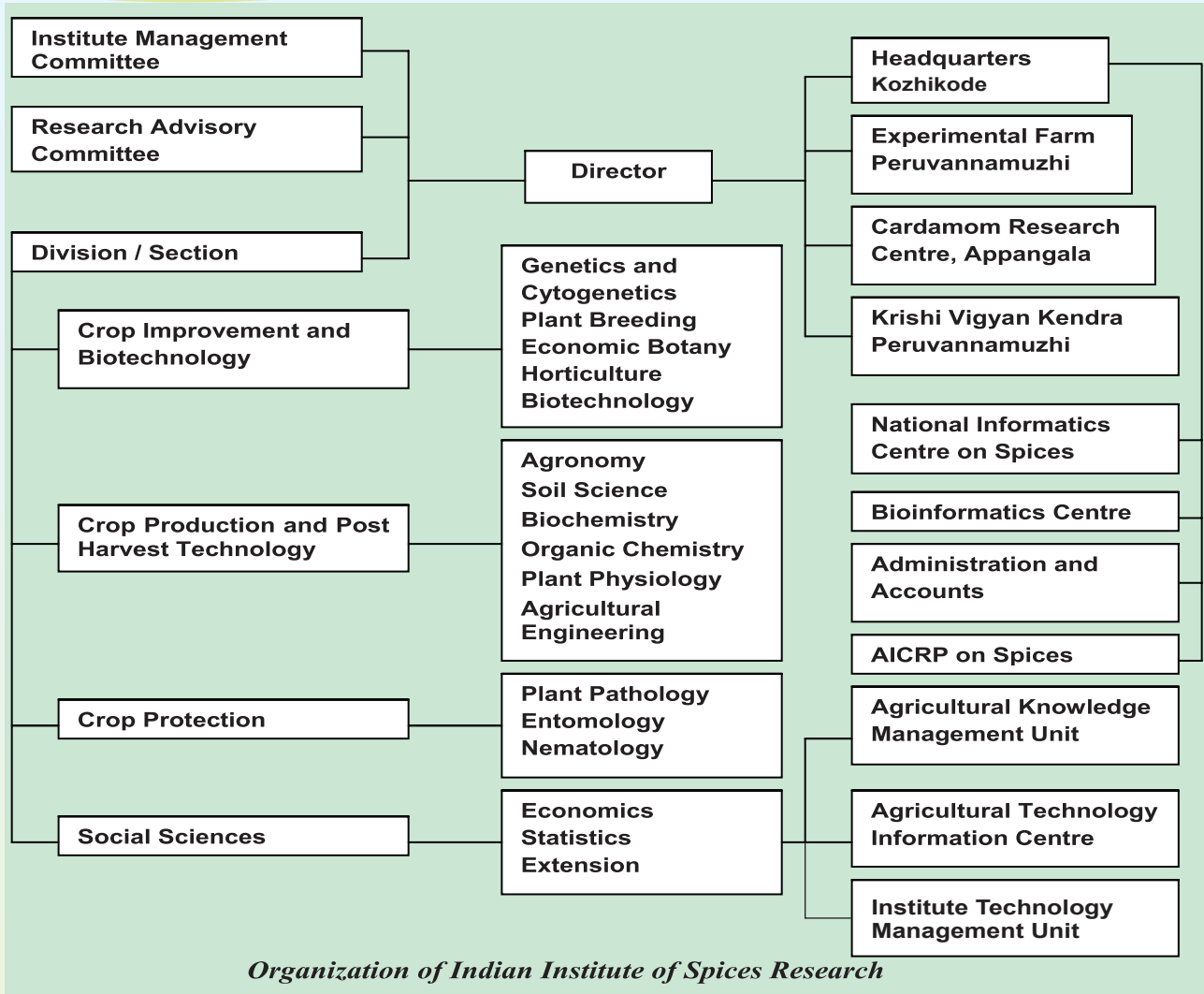
Category	Sanctioned	Position			Total	Vacant
		Kozhikode	Peruvannamuzhi	Appangala		
Scientist	44	28	1	4	33	11
Technical	31	14	10	4	28	3
Administration	24	17	-	2	19	5
Supporting	33	10	7	16	33	-
Total	132	69	18	26	113	19

### Staff position of KVK, Peruvannamuzhi

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







**New facilities**



**Centralized laboratory for advance research**



**Containment green house facility**



**Farm pond**





## PAST ACHIEVEMENTS

**Black pepper:** Germplasm collections obtained over the years through explorations are being maintained at IISR as well as in other alternate sites *viz.*, Appangala and Kidu of Karnataka for developing improved varieties for yield, quality, abiotic and biotic stresses. GIS is being employed to identify species richness. The genetic stock has led to release of improved varieties such as IISR Sreevara, IISR Subhakara, IISR Panchami, IISR Pournami, IISR PLD-2, IISR Thevam, IISR Girimunda, IISR Malabar Excel and IISR Shakthi. Front-line demonstration programme was undertaken using the released varieties in the farmers' field. Some of the unique germplasm have been registered with NBPGR at New Delhi. Two accessions, INGR 8099- *Piper thomsonii* (IC 398863) - for its unique character for sex change and INGR 8100- *Piper nigrum* (IC 563950) - A novel spike variant with proliferating spikes, were registered with NBPGR for their unique characters. 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 *Piper colubrinum* and *P. nigrum* helped in the identification of many genes involved in secondary metabolism. Seedlings of *P. colubrinum* on screening with *P. capsici* showed segregation of the resistance character, 21 plants being resistant to *Phytophthora*, two plants susceptible and the rest showing moderate resistance.

Putative transgenic black pepper plants with osmotin gene conferring resistance to drought and *Phytophthora capsici* have been developed. *In vitro* and *in vivo* propagation methods were standardized. Plantlets developed through micropropagation were established in farmers' field in Kerala and Karnataka. Portion of gene conferring resistance against *Phytophthora capsici* was isolated by targeted gene amplification using degenerate primers from *Piper colubrinum*. The spacing, nutrient and water requirements were standardized for different soil types of pepper growing regions. Irrigating pepper vines once in a fortnight from March to May months at the rate of 50 litres/vine enhanced yield substantially. Vines which

received 200-300  $\mu$  moles  $m^{-2}$   $sec^{-1}$  produced 3.4 kg/vine and those which received around 100  $\mu$  moles  $m^{-2}$   $sec^{-1}$  produced 1.8 kg/vine under Madikeri conditions. 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. 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*.

ARNA 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. 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  $\beta$ -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 $\mu$ l/ml caused 100% mortality of *R. similis* within 24 h. 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 EC<sub>50</sub> and EC<sub>90</sub> 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. 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. ITS region of *R. similis* was amplified with universal primers. PhytoWeb, a comprehensive portal on *Phytophthora* diseases of horticultural crops in India was developed. Phytolib, an electronic database of research publications on *Phytophthora* has also been 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 with the adoption of scientific packages as compared to 620 kg/ha 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 bio control 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:** IC numbers have been obtained for all the available germplasm. The germplasm bearing unique characters have been registered with NBPGR, New Delhi. Improved varieties such as IISR Vijetha, IISR Avinash and IISR Suvasini have been developed. Two of them having mosaic or rhizome rot resistance have been popularized among the farming community. New high yielding genotypes such as APG 293, 398, 416 and 250 are found to be promising. Accessions IC 547146 and IC 349630 were short listed for high yield with more number of capsules per plant. EST Data base searches for sequence information containing microsatellites from ginger revealed 94 SSR candidates. Twenty primers were designed. Characterization of export grade cardamoms from India, Sri Lanka and Guatemala revealed the superiority of Indian produce for the physical parameters such as seed to husk ratio, weight of 100 capsules, number of capsules in 100g, bulk density and moisture content. GC-MS study confirmed superiority of Indian cardamom over Guatemalan and Sri Lankan cardamom. Molecular profiling of Indian cardamom revealed the existence of two genetically distinct clusters such as “Kerala cluster” and “Karnataka cluster” 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. Crosses 893×RR1, GG×RR1, CCS1×GG, GG×893 and CCS1×GG showed more drought tolerance as they took more time to fold leaves (leaf rolling) under open light than other crosses. High quality (more than 40%  $\alpha$ -terpinyl acetate) cardamom accessions such as NHY 14, MB 3, NHY 18 and OP 28 have been identified. Six genotypes namely, IC 547222, IC 547223, IC 349645, IC 349649, IC 547158 and IC 349637, exhibited moderately resistant and highly resistant reactions against leaf blight and rhizome rot. A procedure for total RNA isolation from cardamom and detection of CdMV through reverse transcription–polymerase chain reaction (RT-PCR) using primers designed for the conserved region of coat protein was standardized. Surveys conducted in major cardamom growing areas of Karnataka and Kerala, revealed the prevalence of Banana bract mosaic virus (BBMV) infection. A reliable RT-PCR based method was also developed for detection of the virus in plants.

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 and 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:** Seven hundred accessions are being maintained in field germplasm conservatory. These accessions have been regularly utilized in the genetic improvement programme. An *in vitro* gene bank was established for conservation of germplasm. Three varieties namely, IISR Varada, IISR Rejatha and IISR Mahima were released for high yield and quality. An exotic collection from China supplied by a farmer from Quilon, Kerala showed vigorous plant morphology, variation in flower colour pattern, high pollen fertility and bold rhizomes. Cross specific amplification of rice microsatellites was successfully done in ginger. Ginger Acc. 195, a tetraploid having  $2n=44$ , showed mean pollen fertility of 67.73%

by glycerol-carminum 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 even after three repeated inoculations with *R. solanacearum*. 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. 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 rhizomes indicated that fresh dry rhizomes contained higher level of monoterpenes namely, Z-citral and E-citral whereas the dry rhizomes were predominated by the sesquiterpene hydrocarbons namely, zingiberene, farnesene and sesquiphellandrene. Bacterial wilt pathogen, *Ralstonia solanacearum* in North Eastern states, Sikkim and Kerala were found similar in a molecular fingerprinting indicating strain migration from one place to another. Ginger strain of *R. solanacearum* was found to infect turmeric, cardamom, *C. aromatica*, *C. zedoaria*, *Kaempferia galanga*, *Zingiber zerumbet* and tomato. Indian Mango ginger, *Curcuma amada* was found to be free from bacterial wilt even under inoculated conditions. The species of *Pythium* causing rhizome rot in Kerala, Karnataka, Uttar Pradesh and Sikkim was identified as *P. myriotylum*. Nine actinomycete isolates were found to be antagonistic to *R. solanacearum*. Technique for seed rhizomes treatment (for elimination of bacterial wilt pathogen) and integrated disease management strategy for soft rot and bacterial wilt diseases and shoot borer was developed. 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. 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. 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. Seven high curcumin and high yielding varieties, Suvarna, Sudarsana, Suguna, Prabha, Prathibha, IISR Alleppey Supreme and IISR Kedaram were released for commercial cultivation. Efficient protocol for plant regeneration through organogenesis and somatic embryogenesis was standardized. Variations in rhizome morphology were observed among calli-regenerated somaclones indicating somaclonal variation. Accessions with high curcumin and root knot nematode resistance were identified. About forty seedling progenies with higher curcumin (> 3%) and dry recovery (> 20%) were identified. The natural enemies of shoot borer (*Conogethes punctiferalis*) were documented. Three different curcuminoids (curcumin, de methoxy curcumin and bis de methoxy curcumin) could be separated from oleoresin of rhizomes by employing chromatographic techniques. Oil components have been characterized by GC-MS. A PCR based method was developed to detect adulteration of turmeric powder with wild *Curcuma* species. Partial sequence of *pal* gene was isolated with PCR conditions optimized using *pal* gene specific primers, designed based on sequences available in the public domain. A 522 bp product amplified by PCR was isolated, cloned and sequenced. 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. Organic farming system was developed. 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 Prathiba in AP, Karnataka and TN were studied.

**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  $\alpha$ -phellandrene,  $\beta$ -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. With the help of BIO CLIM models (Altitude and Rainfall) of DIVA GIS, the existence of *Garcinia* in the NE Himalayan states was predicted. 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.

**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* × *V. tahitensis*, *V. tahitensis* × *V. planifolia* and selfed progenies of *V. tahitensis* were established *ex vitro*. Over 1000 seed progenies of *V. planifolia* are being field tested for yield and disease resistance. 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 ICBD-10, Kt-pl-19 and EC-18 were found promising with high colour value and low pungency. PCR based technique was developed to detect adulterants in commercial chilli powder.



# 1. Black Pepper

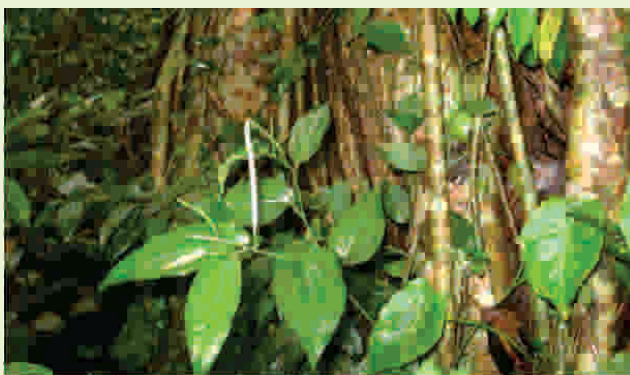


## Crop Improvement

The black pepper germplasm is maintained in the nursery and field genebank. The present status is 2936 accessions (Wild pepper- 1418, Cultivars- 1509, Exotic species- 9). Two hundred and thirty accessions of wild germplasm are conserved at the field genebank at CRC, Appangala. One hundred and thirty accessions were characterized for eight morphological characters. Herbarium specimens of 100 black pepper, 50 turmeric and 50 ginger accessions were prepared and preserved in the herbarium facility. Germplasm collections were carried out from Idukki WLS, Sabari hills and Goodrickal forest range and also from the forests of Kasaragod district. A total of 236 accessions were collected. Endangered species viz. *Piper barberi* and *P. hapnium* were located and collected from Sabari hills. This is the first report of locating *P. barberi* from Sabari hills. A variant of *P. nigrum* with lemonish flavor also collected from this area (Fig 1.1). In addition, two cultivar accessions from Assam were collected and added to the germplasm. The species diversity collected during the period under report is listed in Table 1.1.

**Table 1.1. Species diversity of *Piper* collected and conserved**

Species	No. of accessions
<i>Piper nigrum</i>	106
<i>P. sugandhi</i>	60
<i>P. galeatum</i>	27
<i>P. trichostachyon</i>	23
<i>P. hymenophyllum</i>	10
<i>P. attenuatum</i>	6
<i>P. argyrophyllum</i>	4
<i>P. mullesua</i>	3
<i>P. hapnium</i>	2
<i>P. barberi</i>	1
<i>P. longum</i>	1
<b>Total</b>	<b>243</b>

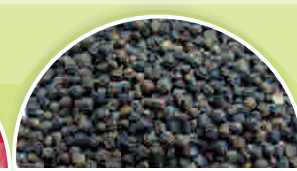


(a)



(b)

**Fig. 1.1 Collections from Sabari hills (a) *P. hapnium* (♂) (b) *P. nigrum* (lemonish flavor)**





### Breeding for biotic and abiotic stresses

The two hundred hybrid progenies from the cross Subhakara × Coll. No. 816 are maintained in the field. Twenty hybrid progenies were screened for pollu infestation and all the progenies were found susceptible. Four hundred seedling progenies were developed from crosses involving Subhakara and two drought tolerant accessions (*viz.* 1495 and 813). About 200 seedling progenies were screened for drought and planted in the field for mapping studies.

One hundred and forty lines from Panniyur 1 × Subhakara crosses were maintained in the field. Data on segregating characters like shoot tip colour, leaf shape and size, length of laterals, spike and fruit characters were recorded. A few lines were found to be promising for high yield. Thirty transgenics were regenerated with Osmotin and five transgenics were multiplied.

### Screening mapping population for *Phytophthora* resistance

Fifty seven lines selected as association mapping population were screened using leaf and stem inoculation methods. The plants were rated as resistant, moderately resistant and susceptible in both leaf and stem inoculation methods and the average rating was taken as disease severity index (DSI) and those with DSI < 30% were rated as resistant, 31- 40% as moderately resistant and > 40% as susceptible. Most of the genotypes were grouped as either susceptible or moderately

resistant. None of the genotypes were found to give resistant reaction. Two genotypes, Acc. 1324 (Aimpiriyan) (Fig 1.2) and HP 780 (Panniyur 1 × Karimunda) gave most tolerant reaction after three rounds of screening. This hybrid was also found to be resistant to *Phytophthora* in earlier screening.



Fig 1.2 Lesion development of Acc. 1324 Aimpirian during leaf and stem screening after 72 hrs

### Amplification of resistance gene candidates using degenerate primers

Three R-gene-specific degenerate oligonucleotide primers that had previously been used in other taxa were selected for amplification of resistance gene candidates and selected set of primers were designed (Table 1.2) according to the conserved P-loop (GGVGKTT) and hydrophobic domain (GLPLAL) from the N, L6 and RPS2 genes of the NBS-LRR class specific against pathogens.

Table 1.2. Specific primers of degenerate R-genes used for screening of NBS sequences analogous to resistance genes in *Piper nigrum* and *P. colubrinum*

Primer code	Sequences (5'-3')	Conserved motif	R-gene/disease resistance protein	References
KgP1F	GGIGGIRTIGGIAARACIAC	NBS-LRR/ PLOOP	RPS2, RPM1, N, and L6	Joshy et al 2011
KgP1R	WTIARIGYIARIGGIARICC	NBS-LRR/ GLPL		
LM637	GGIGGIGTIGGIAAIACIAC	NBS-LRR/ PLOOP	RPS2, N, and L6	Kanazin et al 1996
LM638	ARIGCTARIGGIARICC	NBS-LRR/ GLPL		
Rnbs A-1	TTTGACCTNAACGCNTGG	NBS-LRR/ Rnbs	Disease resistance protein from <i>C. annuum</i> , <i>C. chinense</i> , <i>Nicotiana tabacum</i> , <i>S. demissum</i> and <i>S. tuberosum</i>	
GLPLA-1	GCNAGNGGCAGNCCNCCRCA	NBS-LRR/ GLPL		-

With an objective to know whether there is any difference in sequence among the amplicons some of these (amplified with primer LM637/LM638) were extracted, purified and cloned using pTZ57R/T vector system. The sequence data were subjected to GenBank searches with BLAST and BLASTX algorithm. The amplicon of moderately resistant varieties like IISR Shakthi had 43% identity to *Citrus trifoliata* resistance-like protein and that of P24-O-4 had 73% identity to *Solanum trilobatum* NBS-LRR protein. The susceptible varieties IISR Sreevara and IISR Subhakar had 49% identity to *Solanum trilobatum* NBS-LRR protein and 44% identity to *Mentha longifolia* NBS-LRR like protein, respectively. The nucleotide sequences were translated into polypeptides using the ExPaSy translate tool and identity search was made with the BLASTp algorithm that revealed the presence of NB-ARC domain. Multiple alignment of amino acid sequences were performed using CLUSTALW. The finding of conserved domains viz., kinase-1a, kinase-2 and hydrophobic motif, provided evidence that the sequences belong to the NBS-LRR class gene family. The presence of tryptophan as the last residue of kinase-2 motif further qualified them to be in the non-TIR NBS-LRR subfamily of resistance genes.

### Host - pathogen interactive transcriptome

Transcriptome sequence assembly and analysis was done to facilitate a system-wide approach to study *Piper-Phytophthora* interactions with special emphasis on the identification of genes involved in resistance to the oomycete. The sequencing analysis was done with transcriptomes of *Piper colubrinum* and *P. nigrum* (Var. IISR Shakthi) leaves, challenge inoculated with *Phytophthora capsici*. The transcriptome (mRNA) of *Piper* samples was sequenced with short reads on Illumina Genome Analyzer II platform.

Both *P. colubrinum* and *P. nigrum* transcripts showed maximum hit with *Vitis vinifera* (wine grape) sequences, followed by *Populus trichocarpa* (Poplar) sequences indicating closer relationship of magnoliids with eudicots. Gene ontology assignment programs for functional categorization of those annotated unigenes were done based on similarity with *Arabidopsis*

sequences. In the case of *P. colubrinum* transcripts, 3160 were characterized under molecular functions (GOMF), 5866 under biological process (GOBP) and 2893 under cellular components (GOCC) category. Similarly, *P. nigrum* transcripts were able to map 3469 to molecular functions, 6549 to biological processes and 3419 to cellular component category. The genes involved in other important biological processes such as response to abiotic and biotic stimulus/stress, transport, transcription and signal transduction, were also identified through GO annotations. Broadly, the putative orthologs of genes involved in various pathways and cellular processes were found in both the transcriptomes. Limited number of genes from *P. palmivora* and *P. tropicalis* were also annotated.

A large number of SNPs were also identified in *P. colubrinum* (50972 nos.) and in *P. nigrum* (231680 nos) transcriptome. A variety of transcription factors viz., bHLH transcription factor, bZIP transcription factor, DRE-binding protein, ethylene-responsive transcription factor 2, GAGA-binding transcriptional activator, homeobox transcription factor, MYB transcription factor was found in the transcriptome.

Targeted cloning and sequencing of a resistance gene and transcription factor gene fragment from *P. colubrinum* challenged with *Phytophthora capsici* was revealed to be homologous to similar genes identified in different plant species. The resistance gene fragment cloned earlier (254 bp) was used to BLAST for similar contigs (developed by De novo assembly) derived from the transcriptome of *P. colubrinum*. A contig of 3008 bp, matching with the sequence of the fragment was identified and its further analysis revealed that it has a coding sequence of 913bp amino acids which had high sequence similarity and motifs of plant resistance class of genes.

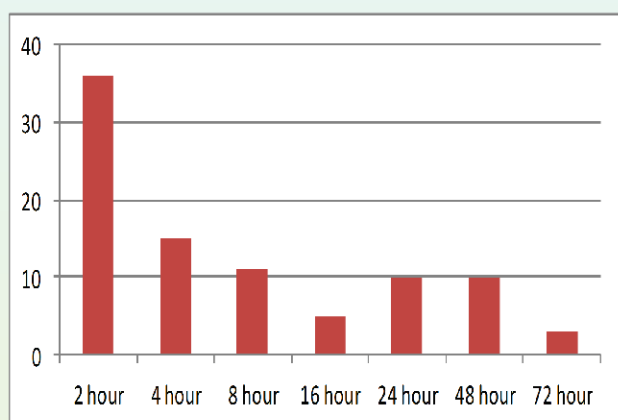
### Gene expression analysis of defense genes using qPCR

Quantitative reverse transcription amplification was carried out to study the expression of two defense related genes viz., osmotin and  $\beta$  1,3 glucanase involved in resistance to *Phytophthora*. *Piper colubrinum* plants challenge inoculated with *Phytophthora capsici* was used in the study at various time intervals of 4 hours, 8





hours, 16 hours, 24 hours, 48 hours, 72 hours and uninoculated leaf sample was used as control. Osmotin gene was found to get induced at approximately 16 hai (hours after inoculation) and maximum induction was reached at 24 hai and declined after that. The expression of  $\beta$  1,3 glucanase genes reached its peak at 48 hpi and dropped at 72 hai. The expression of osmotin was initiated first in comparison to  $\beta$  1,3 glucanase. The expression was minimal at 72 hai for both the genes and may be coinciding with the cell death of HR activity in the inoculated tissues. Real time PCR analysis of R- gene (Fig 1.3) and *WRKY* gene (Fig 4) from *P. colubrinum* were done with leaf samples challenge inoculated with *P. capsici*.

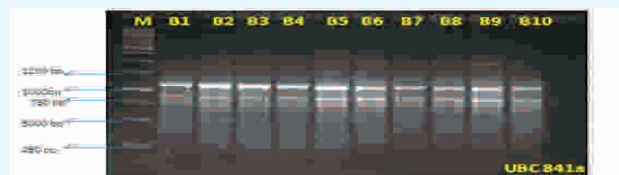


**Fig 1.3. Real Time PCR analysis of the putative R- gene in *Piper colubrinum* challenge inoculated with *P. capsici***

The expression data indicated a peak of expression of R-Gene during the initial stages *ie.* at two hours post inoculation (hpi) and which gradually down regulated upto 16 hpi. A further increase in expression at 24 hpi was also found. The *wrky* gene showed its highest expression at 24 hpi and thereafter it was found to be down regulated.

#### Genetic fidelity testing of TC samples

Ten TC samples of black pepper were received for genetic uniformity testing from TERI, New Delhi. The samples were tested with five ISSR primers and were found to be genetically uniform. (Fig 1.4).



**Fig 1.4. ISSR profile of Black pepper samples using *UBC 841a* primer indicating genetic uniformity**

Lane M : 1 kb ladder ; Lane 1 : B1, Lane 2 : B2, Lane 3 : B3, Lane 4 : B4, Lane 5 : B5, Lane 6 : B6, Lane 7 : B7, Lane 8 : B8, Lane 9 : B9, Lane 10 : B10

#### Performance of grafts

Grafts of Sreekara on *Phytophthora* tolerant lines P-24, C-1090 and *P. hamiitoni* were prepared for planting. Sreekara showed 50% success on *P. hamiitoni* as root stock.

#### Interstock grafting studies

*P. ornatum*, a resistant species was grafted with five *Piper* sp. as potential interstocks since black pepper is incompatible with *P. ornatum*. The species tried were *P. hamiitoni*, *P. chaba*, *P. argyrophyllum*, *P. attenuatum*, and *P. hymenophyllum*. The best success was obtained with *P. hamiitoni* (100%). The other species gave poor success and were susceptible to pathogens. When Sreekara was grafted on *P. ornatum* with *P. hamiitoni* as interstock 80% sprouting was observed and the growth is normal. So the combination *P. ornatum* + *P. hamiitoni* + Sreekara appears promising. However, *P. ornatum* was found highly susceptible to *Sclerotium rolfsii* during rainy season limiting its usefulness. Considering the above weakness, *Piper* sp. Kottakkal (Acc. 5815) earlier identified as resistant but incompatible with black pepper was grafted with nine *Piper* sp to see compatibility as interstock and among them *P. longum*, *P. colubrinum* and *P. hamiltoni* gave 70% success. *P. longum* being susceptible to the fungus, the other two species which are resistant and compatible with black pepper are promising. *Piper* sp. Kottakkal (Acc. 5815) has very vigorous growth and profuse root system free from the pathogens.

#### Allelopathy effect of tree exudates

Results revealed inconsistent effects of tree extracts at varying concentrations. However, irrespective of tree species, plant height decreased at higher concentrations of 50 and 100%. In case



of ailanthus extracts, plant height was maximum at 25% concentration but decreased significantly at 50% and 100% concentrations. Likewise, in case of gliricidia, erythrina and garuga, plant height increased up to 12.5% concentration, but showed inconsistent effects at higher dilutions. Results on number of leaves, root length and fresh weight also followed an identical trend. Results on soil analyses revealed no variations in soil pH. Soil mineral N decreased and Bray P & exchangeable K levels increased or decreased at 50 and 100% concentrations. N, P and K uptake by the plants did not show any marked variation across dilutions irrespective of the tree species.

### Organic farming

Black pepper was grown organically by applying FYM, vermicompost, ash, rock phosphate, *Azospirillum*, *phosphobacteria*, *Trichoderma* and *Pseudomonas* sp. (IISR-6 & 853) in comparison with integrated and chemical management systems. The vines grown under organic system recorded yield (0.83 kg/std) on par with integrated and chemical systems (1.0 kg/std each). Among the varieties IISR Thevam and HP 780 yielded higher yield (1.2 kg/std) under organic management compared to Sreekara. The enzyme activities were higher under organic and integrated management systems as compared to chemical systems

### Economic optimum for nutrient response

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. 1.60/standard for N, Rs. 2.40/standard for P and Rs. 5.40/standard for K.

### Drought tolerance

Among 100 germplasm accessions screened for drought tolerance, Acc Nos. 5621 and 5642 maintained relative water content (RWC) above 70 % and membrane leakage (ML) of 9 % or below after 18 days of stress imposition at a soil moisture level of around 8% (Table 1.3). These accessions were grouped under relatively tolerant category.

### Molecular profiling of *Phytophthora* isolates

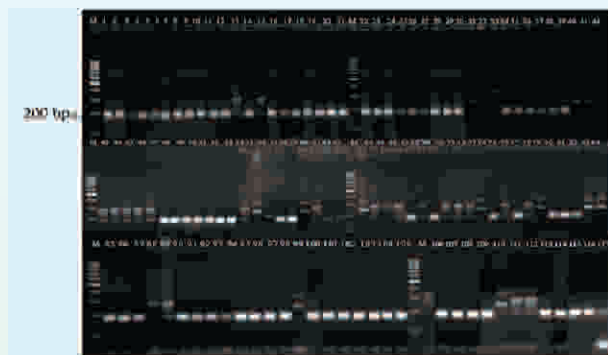
Thirty six SSR primers were designed from the EST database of *Phytophthora capsici* and were screened for their polymorphism among *Phytophthora* isolates. Twenty nine of the 36 primers tested amplified the expected amplicon. Three primers were found to be polymorphic (Fig 1.5) and were used to study the diversity of isolates. A total of 35 alleles were scored. A dendrogram was constructed and cluster analysis was done. The dendrogram showed that the similarity coefficient ranged from 79-100%.

**Table 1.3. Relative water content and membrane leakage values of relatively drought tolerant accessions**

Accession	Days after stress imposition	RWC (%)	ML (%)	Soil moisture (%)
5621	0 (Control)	94.7	4.9	20.5
	6	87.1	7.3	15.6
	18	84.1	8.7	8.4
5642	0 (Control)	91.5	5.5	21.0
	6 DAS	81.4	9.0	15.2
	18 DAS	84.4	8.0	8.0



Cluster analysis using the un-weighted pair-group method with arithmetic averages (UP-GMA) divided the isolates into two major clusters.



**Fig 1.5** SSR profiling of *Phytophthora* isolates from black pepper with primers PC33F-PC33R

M: 100 bp ladder; Lanes 1-117: *Phytophthora* isolates from Black pepper

An attempt was made to use ITS sequencing to identify *Phytophthora* species associated with black pepper. The sequence data of these amplified fragments were obtained using ABI DNA sequencer. BLAST searches for ITS rDNA from these sequences indicated that most of the isolates showed sequence similarity either with *P. capsici* or *P. tropicalis* or both. Two isolates showed sequence similarity with *P. citrophthora*, three with *P. nicotianae*, one with *P. palmivora* and another isolate with *P. infestans*.

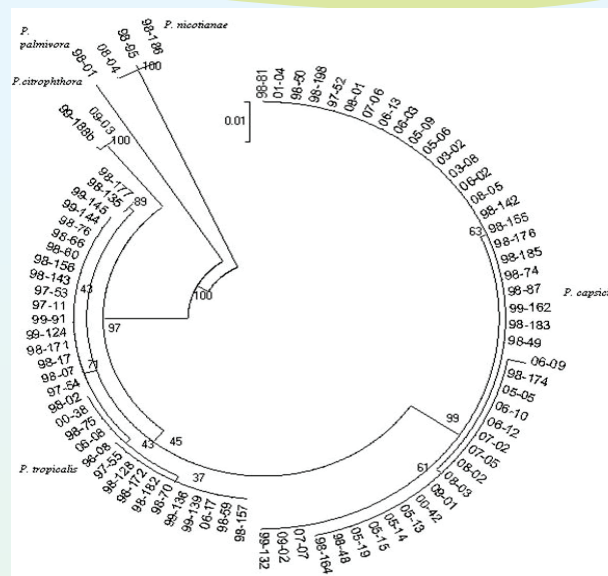
The ITS sequences obtained for different isolates were aligned using the software Clustal W and the maximum likelihood tree was constructed using the software MEGA to understand the similarities between the isolates based on ITS sequence information (Fig 1.6).

### Myco-endophytic fungal association

Myco-endophytic fungal association was confirmed and 70 myco-endophytes were isolated and morphotyped. Eight of the isolates showed *in vitro* antagonism against *P. capsici* and were identified as *Annulohyphoxylon nitens*, *Fusarium proliferatum*, *Daldinia eschscholzii*, *Gibberella moniliformis* and *Ceriporia lacerate*.

### Biocontrol potential of actinomycetes

Actinomycetes isolates from the rhizosphere soil were evaluated against *P. capsici* *in vitro*. The isolates showed high degree of antioomycetal



**Fig 1.6** Maximum likelihood tree of diversity of *Phytophthora* isolates from black pepper by ITS Sequencing

activity against *P. capsici*. The potential isolates were characterized by morphological and molecular tools and sequence information showed that the potential isolates belonged to *Streptomyces* species.

### Evaluation of new chemicals against *P. capsici*, *R. similis* and *M. incognita*

Chemicals such as Fenamidone 10% + Mz 50% (Sectin), Famoxadone 16.6% + Cymoxanil 22.1% (Equation-Pro), Cymoxanil 8% + Mz 64% (Curzate M8), Dimethomorph 50% (Acrobat 50) reported effective against *Phytophthora* species were evaluated *in vivo* against *P. capsici* in comparison with Metalaxyl-Mz. The result showed that Metalaxyl-Mz was comparatively better in preventing foliar infection followed by Sectin 400-450 ppm. Sectin, Equation-Pro, Curzate, and Acrobat were as effective as Metalaxyl-Mz (1250 ppm) in checking the root infection (Table 1.4).

### Integrated management

Endophytic and rhizospheric bacterial biocontrol agents *viz.*, *Pseudomonas aeruginosa* (BP-35, IISR 853), *P. putida* (BP 25), *Bacillus megaterium* (BP 17), *Curtobacterium luteum* (TC10), and *P. fluorescens* (IISR 6) were evaluated under field conditions against foot rot and slow decline disease. The data after four years of planting showed promising results in the treatment with TC10 + Metalaxyl-Mz with increased yield and canopy size when compared to other treatments and was at par with the





**Table 1.4 Efficacy of new fungicides against *Phytophthora capsici***

Treatment	Dosage (ppm)	<i>Phytophthora</i> leaf infection – lesion size in mm (72 h)	Progression of lesion in 7 days	Infection %	<i>Phytophthora</i> presence in soil
Fenamidone 10% + Mz 50% (Sectin)	450	9.00 de	43.5 ab	10	-
Fenamidone 10% + Mz 50% (Sectin)	400	7.00 de	57.90 ab	30	-
Famoxadone 16.6% + Cymoxanil 22.1% (Equation- Pro),	1000	14.1 abc	63.9 a	10	+
Famoxadone 16.6% + Cymoxanil 22.1% (Equation- Pro)	500	11.6 bcd	37.1 ab	30	-
Cymoxanil 8% + Mz 64% (Curzate M8)	3000	13.8 abc	51.9 ab	20	+
Cymoxanil 8% + Mz 64% (Curzate M8)	2500	18.1 a	58.5 ab	0	-
Dimethomorph 50% (Acrobat)	20	16.5 ab	55.9 ab	0	-
Dimethomorph 50% (Acrobat)	10	14.1 abc	52.7 ab	20	+
Ridomil Mz	1250	2.9 e	10.1b	0	-
Control	-	17.3 ab	42.0 ab	40	+

existing recommendation of Metalaxyl-Mz + Phorate.

### Studies on endophytic bacteria

Biochemical and antibiotic sensitivity tests including BIOLOG testing were carried out for endophytic bacteria viz. *Pseudomonas putida* BP25, *Bacillus megaterium* BP17 and *Serratia marcescens* GRB68. Slight inhibition of plant pathogens like *Phytophthora capsici*, *P. tropicalis*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Fusarium oxysporum* etc. was noticed with BP25 and BP17 in dual plate assays. However, GRB 68 showed a higher rate of inhibition of these pathogens.

Genome profiling of *P. putida* and *B. megaterium* was carried out using available whole genome sequences (Table 1.5). The activity prediction of biochemical compounds was done using *in silico* tools. Potential antioomycete secondary

metabolites were identified from *P. putida* through virtual screening and *in silico* docking studies. Similarly around 35 potential metabolites having nematocidal activity were identified from *B. megaterium*.

### Development of Real-Time PCR for detection viruses

A SYBR green based real time PCR for detection of *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV) was developed. Among the two viruses, PYMoV is a DNA virus while CMV is an RNA virus. Real time PCR was performed using template DNA, qPCR master mix and specific primers for each of the viruses. The specificity of the SYBR green assay was evaluated using three different reactions which include virus infected sample, healthy (negative) control and water (without template) control. Results of real time PCR showed strong fluorescent signals only from reactions with

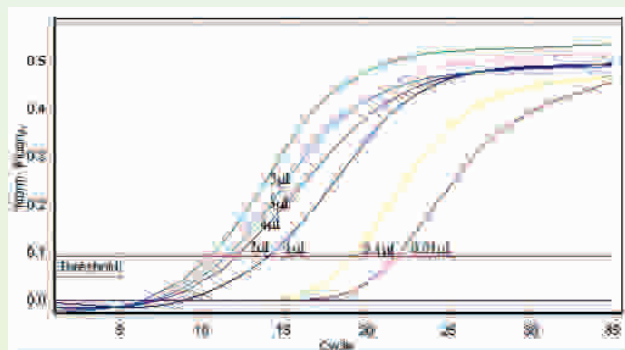


**Table 1.5 Genome profiles of *Bacillus megaterium* and *Pseudomonas putida***

Feature	<i>Bacillus megaterium</i>		<i>Pseudomonas putida</i>			
	DSM 319	QM B1551*	F1	GB-1	KT2440	W619
Genome size (Mb)	5.1	5.5	6.0	6.1	6.2	5.8
Total genes	5272	5805	5403	5529	5516	5309
Protein genes	5124	5629	5308	5433	5420	5212
RNA genes	148	176	95	96	96	97
Pseudogenes	24	17	58	25	70	30
Pathways	262	273	259	253	251	261
Polypeptides	5100	5612	5250	5408	5350	5182
Protein complexes	36	35	7	20	31	16
Compounds	1092	1119	1007	1001	945	1043

\* including plasmids

infected samples, while the signals from healthy sample and water control were superimposed to the baseline under optimized reaction conditions. To test the limits of detection of the viruses, different template volumes viz., 0.01, 0.1, 1.0, 2.0, 3.0, 4.0 and 5.0  $\mu$ l were used. The results showed that SYBR green PCR could detect the presence



**Fig 1.7 Real Time PCR detection of PYMoV in black pepper using different template volumes**

Amplification plots show the peaks and Ct values for different template volumes

of PYMoV in the template volume ranging from 0.01 to 5  $\mu$ l, with Ct values ranging from 22.0-10.0. Results for CMV showed that the real time RT-PCR could detect the presence of CMV in template volume ranging from 0.01  $\mu$ l to 2  $\mu$ l, with Ct values ranging from 17-10. Specificity of the real time PCR reaction was confirmed through melt curve analysis and agarose gel electrophoresis. Melt curve analysis showed a single peak at 83 C and 84 C for PYMoV and CMV real time PCR products respectively confirming specificity of the reaction (Fig. 1.7). Further, agarose gel analysis of the real time

PCR products showed a single band at expected size. The method was validated using large number of field samples collected from different regions and other *Piper* spp such as *P. betle*, *P. longum* and *P. colubrinum*

#### Seed transmission

In order to know the location of the virus in seeds, matured berries collected from healthy and PYMoV infected varieties (Panniyur 1, IISR Thevam and Subhakra) were separated into embryo, endosperm and perisperm. Total DNA isolated from each of these parts was separately subjected to PCR using PYMoV specific primers. Results showed presence of PYMoV in all seed parts in all the varieties tested and the concentration of viral DNA was found to be almost same in embryo, endosperm and perisperm. Out of 1692 germplasm accessions screened for resistance against *Piper yellow mottle virus*, nine accessions belonging to wild *Piper nigrum* and other *Piper* spp showed resistance in the preliminary tests.

#### Development of transgenic plants

Transgenic plants already produced (21 plants transformed with PYMoV sense, 65 plants with PYMoV antisense and 75 plants transformed with CMV sense constructs) were challenge inoculated with respective viruses. Challenge inoculation of PYMoV was done using mealybug, *Ferrisia virgata* while sap inoculation method was used for CMV.

### Anthracnose

Eighteen cultures of *Colletotrichum* isolates from different crops, four from cardamom, four from black pepper, one each from betel vine, ginger, turmeric, clove, elephant foot yam, oil palm, coffee, arecanut, tea and chilli were deposited at National Agriculturally Important Microbial Culture Collection (NAIMCC), NBAIM, Mau and accession numbers were obtained.

Observations on anthracnose incidence in black pepper and weather variables namely, maximum temperature, minimum temperature, number of rainy days and rainfall were recorded during September 2009 to August 2011. It was noticed that the progression of disease followed a low-high-low pattern during months of March 2010 to February 2011. Though the disease was prevalent at lower levels during February (8.33 %), March (7.5%), April (7.5%) and May (14.7%), a rapid increase in the disease incidence was noticed in the month of June (22.08%) and subsequently registered a peak in the month of September (39.1%). Among the temperature variables  $T_{max}$  had a negative correlation (-0.90\*) and  $T_{min}$  had a positive correlation (0.20) with the disease progression. Disease incidence was also found to be the maximum during the months which had

more number of rainy days and it is inferred that both rainfall (0.58\*) and number of rainy days (0.65\*) have positive correlation with the disease progression (Fig 1.8).

Survival of *C. gloeosporioides* infecting cardamom and black pepper was studied under lab, pot and field conditions. The fungus survived in the infected plant debris (Table 6) for about three months under field conditions. Under laboratory conditions the fungus survived for more than four months. The possible role of microsclerotia in the survival of *C. gloeosporioides* was investigated. Microscopical examination of necrotic lesions on runner shoots collected from the field (Fig. 1.9) revealed the presence of dark, melanized, round structures in the tissue. Isolation from the necrotic region yielded typical colony of *C. gloeosporioides* in PDA medium.



Fig 1.9 Necrotic lesions on runner shoots

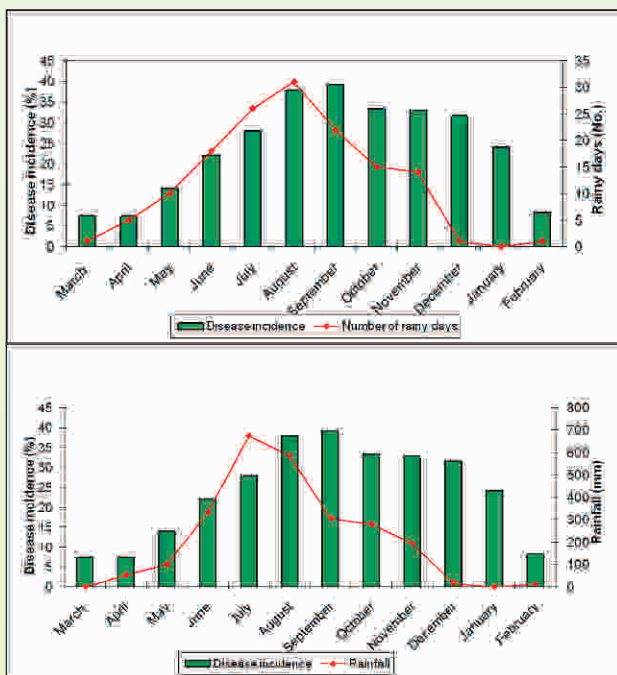


Fig 1.8 Incidence of anthracnose in black pepper and weather variables

In order to produce microsclerotia under lab conditions, the fungus culture was inoculated on nutrient broth. The microsclerotia were predominantly formed on the aerial surface as congregations and entangled in the mycelial. For the production of microsclerotia under *in vitro* conditions and for their easy separation into single units a simple method was devised. For this, conidial suspension was prepared in 2% sucrose solution and subsequently spread on glass slides. These were incubated in moist chamber under ambient temperature conditions. Formation of microsclerotia was observed 7 – 8 days after the incubation period.

Various events in the formation of microsclerotia were investigated. The events included, germination of the conidia, formation of conidial anastomosis tubes (CAT) and subsequent hyphal interaction, aggregation of hyphae and ultimately the formation of melanized round structures.





Three types of microsclerotial germinations were observed under lab conditions *viz.*, sporogenic (production of conidial mass), myceliogenic (production of hyphae) and both sporogenic and myceliogenic germinations.

### Integrated disease management

Three locally available materials (cardamom trash, coffee husk and cowdung singly and in combinations) were tried for mass multiplication of *Trichoderma harzianum* (WYD T11). Initial results indicated that combination of cowdung and coffee husk was the most suitable substrate, which could retain a mean cfu of  $>10^{10}$  for more than five months.

Three noded cuttings were prepared from the runner shoots exhibiting linear necrotic lesions. The cuttings were immersed in different fungicide solutions for 30 minutes. Disease incidence on newly emerged leaves were recorded six weeks after planting (45 days) and repeated at seven days interval for a period of one month. The newly emerged leaves in cuttings treated with Carbendazim + Mz (0.1%) were free from the disease when compared with other fungicides (Fig 1.10). The treatment also delayed disease development by 15 days.

Four fungicides (Carbendazim, Hexaconazole, Carbendazim + Mz and Bordeaux mixture) one plant product (neem based) and an isolate of *Trichoderma harzianum* (WYD T 11) were evaluated under field conditions. Initial observations of field trial indicated that spraying with the combination product of Carbendazim + Mz was found to be promising in managing anthracnose.

### Pollu beetle

The total essential oils and phenols were estimated in berries and leaves of three pollu beetle (*Lanka ramakrishnai*) resistant (816, 841 and 1114) accessions and one susceptible (Panniyur 1) accession. The total essential oil in dried immature berries of susceptible accession (Panniyur 1) was 3.98% and in resistant accessions it ranged from 2.80 to 8.60%. The total phenol content in susceptible accession was 0.613 mg/g and it varied from 0.735 to 1.011 mg/g of

dried immature berries in resistant accessions. The immature berry surface wax in the resistant accessions varied from 0.4782 to 0.6917  $\mu\text{g/g}$  of fresh berries and in susceptible accession it was 0.2926  $\mu\text{g/g}$  of fresh berries. The surface wax content of berries in resistant and susceptible accessions was significantly different



Fig 1.10 Evaluation of fungicides in nursery against anthracnose

### Phenyl propanoids on *Radopholus similis*

Additional 15 phytochemicals from phenylpropanoid pathway of black pepper have been docked to modeled  $\beta$ -1,4, endoglucanases (EGase) enzyme of *R. similis* to assess their binding affinity and consequently their inhibitory activity (Table 1.6). Based on binding energy scores such as moldock score and re-ranking, maximum inhibitory activity was found in Syringin followed by sinapaldehyde and sinapic acid.

### In planta bioassay

A greenhouse trial has been laid out with the most promising compound, ferulic acid, using black pepper *R. similis* infested rooted cuttings. Ferulic acid @ 250 and 500 ppm significantly reduced *R.*



*similis* population in black pepper rooted cuttings. Besides, the mortality of the nematode infested pepper plants was significantly reduced from 70% in non treated plants to 20% in ferulic acid treated plants. Tissue localization of phenylpropanoids was done by treating sections with 0.25% (w/v) diphenylboric acid 2-

aminoethyl ester (DPBA, Sigma-Aldrich) and visualizing immediately with an epifluorescence microscope equipped with a FITC filter (excitation 450–490 nm, suppression LP 520 nm, blue light). Fluorescence of different DPBA-conjugated phenylpropanoids was studied.

**Table 1.6 Docking results of phenyl propanoids against GHF5 endo 1, 4 beta glucanase of *Radopholus similis***

Phytochemical	Mol. wt. (g/mol)	Dock score (kg/mol)	Rerank score (kg/mol)	No. of H bonds	Total interaction (kg/mol)
Syringin	372.37	-98.26	-91.14	10	-128.44
Sinapaldehyde	208.21	-92.81	-89.18	4	-100.41
Sinapic acid	224.21	-91.72	-86.08	6	-99.24
Scopolin	354.31	-90.42	-81.30	8	-131.12
Caffeoylquinic acid	354.31	-86.89	-79.49	11	-135.35
Spermidine	145.25	-85.94	-74.68	6	-91.90
Salicylic acid	138.12	-69.47	-61.33	5	-78.15
Coumaric acid	568.78	-67.31	-59.44	2	-88.30
Coniferin	342.34	-63.99	-54.93	10	-99.69
Lantanilic acid	554.80	-57.60	-51.65	2	-64.19
Oleanolic acid	456.70	-49.91	-39.36	2	-99.55
n- vanillyl nonanamide	293.40	-46.52	-34.67	3	-80.65





## 2. Cardamom



### Maintenance of germplasm

Four hundred and fifty nine accessions have been maintained in the field gene bank (Malabar- 278, Mysore-73, Vazhukka-63 and others-28). During the year the gene bank was enriched with 17 new accessions from RRS, Mudigere.

### Characterization of germplasm

Morphological characterization has been recorded in 50 accessions raised in the field gene bank. Based on the characterization and screening against natural incidence of diseases, the following accessions were shortlisted for future comparative yield trials (CYTs).

- IC 547146 (Yield)
- IC 547161 (yield)
- IC 547147 (Oil and Yield)
- IC 349646 (Leaf blight resistance)
- IC 547223 (Rhizome rot resistance)

Analysis of four successive crop yields of F<sub>1</sub> hybrid progenies of Preliminary Evaluation Trial-I and II resulted in identifying the high yielding hybrids viz., NKE-12 × MB-5 (1499 kg/ha), MB-5 × NKE -19 (1461 kg/ha), GG × NKE-12 (1350 kg/ha), RR-1 × CCS-1 (1245/868 kg/ha), CCS-1 × RR-1 (1022/765 kg/ha), ASH (1930/1119 kg/ha), NKE-12 × GG (1746/741 kg/ha), GG × NKE -19 (1635/833 kg/ha).

### Generating microsatellites

About 270 clones from genomic DNA were isolated and sequenced using hybridization – enrichment method. Twenty four primers were designed and 20 primers giving good amplification were developed. The sequence information obtained from over 200 clones from small cardamom and about 100 clones from large cardamom are being processed for developing primers for more SSRs.

In an effort to identify SSR markers for small and large cardamom 12 rice, 30 ginger and 35 turmeric SSRs were screened for cross generic

amplification. Of these, four rice, eight ginger and seven turmeric SSRs worked with small cardamom. Molecular characterization of 95 accessions of germplasm using 16 microsatellite markers was completed. Molecular profiles were developed for 150 accessions using 25 ISSR markers and 50 accessions of large cardamom germplasm using nine ISSR markers for studying the genetic diversity.

To support molecular data for developing core collections, data on 46 taxonomically important morphological characters based on IPGRI cardamom descriptor (IPGRI, 1994) and DUS guidelines (PPV & FRA, 2009) were also collected in 100 accessions

### Genetic fidelity testing

Ten TC samples were received for genetic uniformity testing from TERI, New Delhi. The samples were tested with five ISSR primers and partial instability was observed in three lines with one of the five primers tested.

### Stress physiology

Twelve short listed genotypes and three checks were field tested for stress and growth, yield and physiological parameters (relative water content and specific leaf weight). Soil moisture content ranged from 15-17% under stress treatment. Relative water content percent ranged from 10.77 (APG 224) to 35.72 % (IC584071) with a mean of 22.35. Specific leaf weight (mg/cm<sup>2</sup>) ranged from 4.11 (Appangala 1) to 5.93 (APG 224) with a mean of 5.25. Stomatal count (40x) ranged from 10.72 (IC584070) to 20.27 (IC584073) with a mean of 12.97. Gas exchange parameters were not consistent. Excised leaves were exposed to direct sunlight, time taken to leaf folding and wilting was recorded. Few genotypes with larger leaves took longer time to fold compared to Malabar types like Appangala 1. Growth and yield parameters were generally reduced under stress.



Total number of tillers per clump ranged from 20.4 (IC 584059) to 40.7 (GG x NKE12) with a mean of 31.2 in control and under stress it ranged from 16.6 (IC 584059) to 29.5 (GG x NKE12) with a mean of 26.3. GG x NKE12 recorded maximum reduction in total tillers number under stress and minimum reduction was observed in IC 584060 (3.44%).

Fresh capsule weight (g/clump) ranged from 520.83 (IC 584059) to 1500 (IC 584079) with a mean of 968.12 g/clump in control and in stress it ranged from 290.83 (IC 584059) to 975.83 (IC 584079) with a mean of 729.2 g/clump. Maximum yield reduction under stress was observed in IC 584079.

Two genotypes recorded better growth and yield parameters. IC 584058 (APG 474) recorded early yield, good setting and bold capsule. It recorded 294 cm plant height, 32 number of total tillers per clump, 22 number of yielding tillers per clump, 44 number of panicles per clump, 92 cm panicle length, 176.8 number of capsules per panicle and 3889.6 capsules per clump with more than 80% bold capsules (>8 mm) (Fig 2.1).



**Fig. 2.1 IC 584058 (APG 474)**

IC 584078 (GG x 893) a multibranched panicle genotype recorded 393 cm plant height, 34 tillers per clump, 28 yielding tillers per clump, 85 panicles per clump, 189 cm panicle length, 71.2 capsules per panicle, 6072 capsules per clump with more than 50 % bold, green capsules (Fig 2.2).



**Fig. 2.2 IC 584078 (GG x 893) – a multibranched type**

### Evaluation for quality

Sixty nine accessions were evaluated for quality and the oil content in the accessions ranged from 2.4-5.0%. GG and IC 547184 were superior with 5% essential oil per 100g capsules. GG and IC 547184 contained 20.8% and 24.1% 1,8- cineol and 48.1% and 49.8%  $\alpha$ -terpinyl acetate respectively. These two accessions showed similar composition except that GG contained relatively higher levels of limonene and geraniol when compared to IC 547184.

### Viruses

Inoculation studies using aphids as well as by sap inoculation were performed at two locations viz., CRC, Appangala and IISR, Kozhikode. The sap as well as aphid inoculated plants did not develop any visible symptoms. The absence of virus in the inoculated plants was further confirmed by real-time PCR.

Seed transmission studies were carried out using immature capsules, immature and mature seeds collected from the cv. Njallani Green Gold. In RT-PCR, all the samples showed a clear amplification of 950 bp for BBrMV and 1050 bp for CdMV, indicating the presence of the viruses in the plant parts tested. Grow-out test was also carried out with the seeds collected from infected capsules. The seeds were sown in separate seed pans and kept in insect proof glass house. All the seedlings were asymptomatic and the absence of both the viruses in the seedlings was confirmed by performing RT-PCR (Table 2.1).





**Table 2.1. Details of seed transmission study of CdMV and BBrMV**

No. of seeds sown	No. of seeds germinated	Days taken for germination	% germination	Symptomatic seedlings	No. of seedlings tested	No. of PCR + <sup>ve</sup> seedlings
300 (CdMV)	245	21-26 days	81.7	0	70	0
250 (BBrMV)	200	25-28 days	80.0	0	50	0
100 (Healthy)	82	21-26 days	82.0	0	10	0

### RT-PCR based detection

The specificity of SYBR green assay was evaluated using three different reactions which included infected sample, healthy (negative) control and water (without template) control. In order to check the influence of RNA extraction method, real time assay was performed with RNA extracted by manual and kit methods separately.

The PCR products were analyzed by agarose gel electrophoresis. The assay with CdMV and BBrMV sample displayed the expected band of 127 bp (CdMV) and 117 bp, whereas healthy and water control did not amplify (Fig. 2.3).

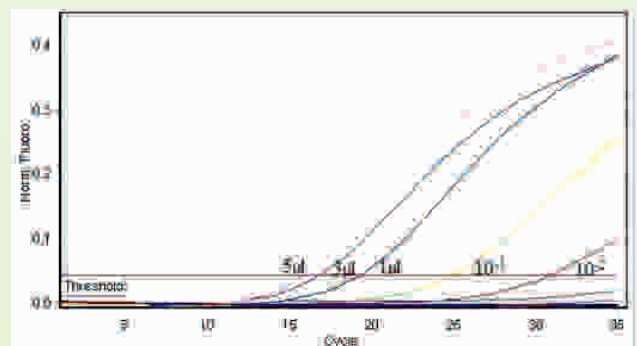
**Fig. 2.3 Amplified products of real Time PCR on agarose gel**

M- 100bp ladder, Lane 1-BBrMV isolate from Appangala, Lane 2- BBrMV isolate from Idukki, Lane 3- CdMV isolate from Appangala, Lane 4- CdMV isolate from Idukki, Lane 5-Healthy control (using BBrMV specific primers).

The sensitivity of SYBR green assay was tested using different template RNA volumes *viz.*, 0.01, 0.1, 1.0, 3.0 and 5.0  $\mu$ l template for RNA isolated by manual method and 0.005, 0.01, 0.1, 1.0, 3.0, 5.0, 7.0 and 10.0  $\mu$ l template for RNA isolated by kit method. For manual method, the SYBR green

RT-PCR could detect the presence of BBrMV in the infected sample in the tested range of 0.01  $\mu$ l to 5  $\mu$ l, with Ct values ranging from 15.78-32.12. In the case of CdMV, the SYBR green RT-PCR was unable to detect the virus at a template volume below 1  $\mu$ l while virus could be detected in template volumes ranging from 1 to 5  $\mu$ l with Ct values from 26.0 to 20.0.

For kit method, the SYBR green RT-PCR could detect the presence of BBrMV in the infected sample in the tested range of 0.005  $\mu$ l to 10  $\mu$ l, with Ct values ranging from 14.60-27.14 (Fig 2.4). In the case of CdMV, the SYBR green RT-PCR was unable to detect the virus at a template concentration below 0.1  $\mu$ l. However, the real time assay with template isolated by manual method could detect the virus at a minimum concentration of 1  $\mu$ l.

**Fig. 2.4 Real Time PCR detection of BBrMV infected cardamom sample using different template volumes.**

Amplification plots show the sensitive threshold as 0.1  $\mu$ l with a Ct value of 31. The water control and healthy control did not result in any signals

### Rhizome- Root rot

Surveys were repeated in Wayanad and Idukki districts of Kerala, Hassan and Kodagu districts of



Karnataka to study the seasonal variation of rhizome and root rot diseases. Incidence and severity of rhizome rot disease was higher in Meppadi Panchayat of Wayanad, Kerala and Appangala and Kadagudalu area of Karnataka. About 85 isolates of fungi were isolated from the samples of rhizome and root rot disease. The fungi included *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium* spp, *Colletotrichum* sp, *Pythium vexans*, *Botryodiplodia theobromae* and unidentified cultures.

Morphological characterization of 10 *Fusarium* and eight *Rhizoctonia* isolates has been completed. 45 isolates of *Trichoderma* spp isolated from Karnataka, Kerala and Tamil Nadu have been characterized based on morphological characters. Pathogenicity of 10 *Fusarium* isolates was tested on IISR Kodagu Suvasini. Among the isolates, Appangala isolate caused complete wilting of the seedling within six days after inoculation.

### Leaf blight

Four fungicides (Carbendazim, Hexaconazole, Carbendazim + Mz and Mz) and the one plant product (neem based) and an isolate of *Trichoderma harzianum* (WYD T 11) were evaluated under field conditions. Initial observations indicated that spraying with hexaconazole was promising in managing leaf blight.

### Thrips

Three hundred and sixty lines from the germplasm block at CRC, Appangala were screened against thrips (*Sciothrips cardamomi*) in the field. Eighteen accessions had damage below 10% with the lowest damage on IC 379596 (5%). The capsule damage was more than 90% on 22 accessions. Accessions IC-349457, IC 349350 and GG recorded 100% capsule damage.

### Evaluation of insecticides

Evaluation of insecticides and natural products including new molecules was done on the var. CCS1. The sprays were imposed once in February, March, April, July, and September. The trial indicated that at first harvest, all treatments except neem soap and Thiocloprid were significantly superior in reducing the damage.

All the other treatments were at par with each other. At second harvest, all treatments except Dinotefuron were significantly superior in reducing the damage when compared with control (15.80%). The lowest damage was observed in plants treated with Fipronil (0.63%) which was on par with Thiomethoxam (1.25%), Imidacloprid (1.93%), Spinosad (2.07%), Zolone (3.36%) and Thiocloprid (3.37%) (Table 2.2).

**Table 2.2. Evaluation of insecticides and natural products for control of cardamom thrips**

Treatment	Per cent capsule damage*	
	Second harvest	First harvest
T1- Neem soap	7.64 ab	9.63 bc
T2- Spinosad	0.00 b	2.07 de
T3- Vertemec	5.18 b	4.28 cd
T4 - Thiamethoxam	0.30 b	1.25 de
T5- Thiocloprid	6.47 ab	3.37 de
T6- Imidacloprid	0.10 b	1.93 de
T7- Cyhalothrin	3.88 b	7.19 cd
T8- Zolone	3.08 b	3.36 de
T9- Fipronil	0.89 b	0.63 e
T10- Quinalphos	2.09 b	5.03 cd
T11- Dinotefuron	5.00 b	13.23 ab
T12- Control (Water spray)	16.20a	15.80 a

\*Average of three replications. Mean separation in a column by DMRT at 5% Level; Means with same letter are not significantly different.



# 3. Ginger



## Genetic resources

Six hundred and fifty nine accessions have been maintained in the field gene bank. Wild germplasm exploration and collection were carried out in the Thekkady forests, Idukki district, Kerala. A putative wild type (*Zingiber officinale* (Wild)) was collected and conserved. The collection is characterized by very small rhizomes, reduced tillers, medium stature and less abundance (Fig 3.1).



Fig 3.1 *Z.officinale* collected from Thekkadi forests

## Evaluation of nematode tolerant ginger accessions

A trial was laid out with four accessions along with check, IISR Varada and evaluated during 2009-12. Morphological and yield characters were recorded. Among the four nematode tolerant accessions, mean yield per bed (kg) over three years ranged from 5.72 to 9.55. Maximum yield was recorded in Acc. 219 followed by IISR Varada (Table 3.1).

**Table 3.1 Evaluation of nematode tolerant ginger accessions for yield**

Entries	Yield (kg/3m <sup>2</sup> )			
	2009-10	2010-11	2011-12	Mean
Acc. 372	6.5	6.67	3.99	5.72
Acc. 65	6.0	7.33	4.03	5.79
Acc. 219	12.0	11.00	5.64	9.55
Acc. 251	6.0	8.67	3.96	6.21
Varada	-	10.63	5.07	7.85
Mean	-	8.59	4.60	
SEd	-	0.62	0.56	
CD (0.05)	-	1.33	1.24	
CV (%)	-	10.24	14.76	

Pooled analysis of the yield and quality data of Nepal accessions indicated the superiority of Accs. 578, 581 and 593 (Table 3.2).

## Pollination and embryological studies

*In vivo* pollination was performed between the high pollen fertile tetraploids (2n=44) viz., Acc 195 and Acc.821 identified earlier. A total of 28 flowers were pollinated till date. No fruit set was observed in self as well as cross pollinations. 20 MC6 generation plants from the mutant population were checked for chromosome number. All of them had the normal chromosome number of 2n=22. One hundred and sixteen MC6 and 181 MC5 generation plants of irradiated varieties were maintained in pots/bags. Samples of all these material were screened against *Ralstonia solanacearum*. After three rounds of inoculation two mutants viz., R 0.9-14 and M 0.5-1(1) were reported as surviving the treatment.

## Organic Farming

Ginger was grown organically by applying FYM, vermicompost, ash and rock phosphate, Azospirillum and phosphobacteria and *Trichoderma & Pseudomonas* sp. (IISR-6) in comparison with integrated and chemical management systems. The management systems (organic, integrated and chemical) yielded on par in all the varieties (Varada, Rejatha and Mahima) studied. Application of FYM + Neem cake + Vermicompost yielded highest on par with FYM + Panchagavya application. The soil enzyme levels were found to be higher under integrated and organic systems than chemical management system. Integrated management recorded significantly higher oil and varieties (Varada, Rejatha, Mahima) were on par under different management systems. Oleoresin content was significantly higher under organic management in Varada (4.5%).

## Economic optimum for nutrient response

Targeted yield equations for predicting nutrient



**Table 3.2 Pooled analysis of mean yield per bed and mean quality traits**

Acc. No	Mean yield (3m <sup>2</sup> )	CF (%)	Oleoresin (%)	E.oil (%)	Dry recovery (%)	Mean dry yield (3m <sup>2</sup> )
552	10.15	2.96	3.80	1.2	21.13	2.14
553	10.90	3.33	3.73	1.2	18.80	2.05
573	10.19	2.90	3.45	1.2	19.90	2.03
574	09.71	2.03	3.67	1.0	21.20	2.06
578	10.99	2.67	3.30	1.2	20.50	2.25
581	11.93	2.13	3.57	1.2	19.70	2.35
589	09.82	3.20	3.47	1.2	19.07	1.87
591	10.31	2.67	3.53	1.2	20.77	2.14
592	10.14	3.03	3.27	1.6	19.67	1.99
593	11.73	2.30	3.20	1.6	18.17	2.13
597	09.76	2.93	3.63	1.2	20.40	1.99
598	09.62	2.20	3.63	1.2	20.40	1.96
Varada	10.53	3.35	4.56	1.3	19.25	2.03
CD (p.05)	1.12	-	-	-	-	
CV (%)	17.32					

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 ₹ 3.75/bed for N, ₹ 1.30/bed for P and ₹ 0.60/bed for K.

### Source sink relationship and endogenous hormone levels in varieties

Partitioning studies in three different varieties viz., IISR Varada, IISR Rejatha and IISR Mahima showed that at 50 days after planting (DAP), shoots received 70.8 - 74.5% biomass and rhizomes received 18.5 - 21% while at 150 DAP shoots received 23 -33.4% and rhizomes received 63.3 - 74.2% of the total biomass. Among the varieties, IISR Varada showed maximum partitioning of dry matter in to rhizomes and minimum in to root and shoot compared to other varieties at 150 DAP (Table 3.3).

At 50 DAP, rhizomes had only 5.6 - 6.6 % starch while at 150 DAP, rhizomes accumulated 49 - 56.2 % starch. Among the three varieties, IISR Varada showed highest photosynthetic rate (9  $\mu$  moles m<sup>-2</sup> s<sup>-1</sup>) and the biomass accumulated in rhizomes was also more in IISR Varada (74 % at 150 DAP). Quantification of endogenous auxins and cytokins in rhizomes revealed that auxin

(44.6 - 66.8 pico moles) and cytokinin (18.5 - 31.2 pico moles) accumulation was maximum during rapid rhizome development (150 DAP) period.

**Table 3.3 Biomass partitioning in ginger**

	Total DW/ plant (g)	Shoot (%)	Rhizome (%)	Root (%)
<b>IISR Rejatha</b>				
50 DAP	3.36	74.47	18.47	7.06
100 DAP	30.67	49.76	45.83	4.41
150 DAP	73.20	28	67.5	4.4
<b>IISR Suprabha</b>				
50 DAP	4.70	71.65	21.38	6.97
100 DAP	28.55	44.34	51.29	4.37
150 DAP	39.00	33.40	63.20	3.60
<b>IISR Varada</b>				
50 DAP	4.54	70.80	20.99	8.21
100 DAP	37.73	47.36	48.80	3.84
150 DAP	67.80	23.00	74.20	2.80





**Table 3.4 Effect of PGPR on ginger sprouting and soft rot disease incidence in green house**

Treatment	Sprouting (%)	Soft rot (%)
GRB 35	100.0	50
GRB 68	100.0	50
GRB 35 + GRB68	91.6	50
Metalaxyl mancozeb	80.0	80
Control	64.2	100
LSD	30.0	23

### Rhizome rot

Treatments included selected three strains (GRB 35-*Bacillus amyloliquifaciens*, GRB 68-*Serratia marcescens* and IISR 51-*Pseudomonas*), one bactericide-Streptomycin, one fungicide-Metalaxyl-Mz and a control. Out of the two sets of experiment, one set of pots were arranged for evaluation against *Ralstonia solanacearum* and another for *Pythium*. Rhizomes were treated with 1% starch solution containing bacterial suspensions ( $\sim x 10^{10}$  cfu mL<sup>-1</sup>) for one hour, shade dried for 24 hours and planted @ two rhizomes (25 g) per pot. The booster dose was applied during in three regular intervals (30, 60 and 90 days of planting, DAP). The sprouting count was recorded on 30 DAP (Table 19). The pathogen was inoculated after one month of planting. The disease incidence and sprouting results (Table 19) indicated that *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB 68) were effective in disease control and sprouting of ginger. Field experiment also revealed that *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB 68) were effective for disease control and plant growth promotion compared to a consortium of GRB 35 and GRB 68, chemical control (Metalaxyl- Mz) and control (Table 3.4).

### Bacterial wilt

MLST analysis of five housekeeping genes dispersed in the chromosome, and three virulence-related genes located on the megaplasmid was done to classify *R. solanacearum*. PCR amplification of

housekeeping genes (*ppsA*, *adk*, *gapA*, *gdhA*, *gyrB*) and virulence genes (*hrpB*, *fliC* and *egl*) in 21 strains of *R. solanacearum* was compared. Allele numbers were obtained by sequence comparison with alleles documented in the database [www.pamdb.org](http://www.pamdb.org). Several novel alleles could be found in ginger strain of *Ralstonia solanacearum* showing the diversity within the biovar of ginger *R. solanacearum*. rec N, a gene coding for DNA repair protein, was used for phylogenetic analysis of *R. solanacearum* representing different hosts and geographical locations in India and it was found that rec N can be used as a tool for classifying *R. solanacearum* into different Phylogentic groups. LAMP (Loop mediated isothermal amplification) method was partially standardized for the detection of *R. solanacearum* in soil.

### Evaluation of biocontrol agents

Bio-control experiments for bacterial wilt management using bacterial antagonists (*P. fluorescens* SBW25, *P. fluorescens* PF5, *P. fluorescens* Phz, *P. aeruginosa* IISR 51, *Stenotrophomonas maltophilia* GEB13, *Acinetobacter calcoaceticus* GEB 19), actinomycetes (*Streptomyces* sp) and antagonistic plants (*Tagetes patula* and *Ocimum sanctum*), showed promising result with pre-planting of *Tagetes patula*.

Actinomycetes isolated from the rhizosphere of healthy ginger plants were evaluated against *R. solanacearum* both *in vitro* and *in planta*. Among the 24 isolates, nine isolates showed *in vitro* inhibition whereas only one isolate (Act 4) showed promising *in planta* inhibition by reducing the disease incidence to 79% when compared to control. The isolates were characterized morphologically and by molecular methods by *rpoB* (RNA polymerase beta subunit) gene sequencing. Sequence information showed that all the potential isolates belonged to *Streptomyces* species. One isolate was identified as *Kitasatospora setae*.

### Shoot Borer

Leaf cuticle wax and total phenols were estimated in mature leaves of seven resistant (Accs. 171,203, 227,247, 252, 260, and 432) and five susceptible (Acc. Nos.137, 191, 208, 480 and 495) accessions. The wax content in susceptible and resistant accessions ranged from 0.0050 to 0.0067 and 0.0054 to 0.2800 mg per 200 cm<sup>2</sup> respectively. The total leaf phenol content in



susceptible and resistant accessions varied from 2.08 to 3.20 and 2.88 to 4.46 mg/ g of dried leaf respectively.

Leaf cuticle wax was estimated in mature leaves of six resistant (Accs. 422,435,589,687,954 and 1026) and three susceptible (Accs. 924, 925 and 1007) accessions. The wax content in susceptible and resistant accessions ranged from 0.0058 to 0.0086 and 0.0055 to 0.3311 mg per 200 cm<sup>2</sup>, respectively.

### Entomopathogenic nematodes

Multiplication of infective juveniles of eight isolate of EPNs in different artificial media viz., Wouts medium, Egg yolk medium, Dog biscuit medium, agar agar medium and wheat flour medium were tested. Maximum number of

infective juveniles of EPN-IISR 06 and EPN-IISR 01 produce in Egg yolk and Wouts medium, respectively (Fig 3.2). Very poor multiplications of EPNs recorded in wheat flour medium. No multiplication was observed in agar-agar and dog biscuit medium.

Two EPN isolates *Steinernema abbasi* (NBAIL SA 01) and *Heterorhabditis indica* (NBAIL Hi 1) were tested for infectivity against larva and pupa of shoot borer *Conogethes punctiferalis* under laboratory conditions. Both species were found to be pathogenic against shoot borer larvae, causing 100% mortality within 48 h, whereas only 17 and 33 % mortality was found against shoot borer pupa. One species, *Oscheius gingeri* was identified as new species on the basis of morphological and molecular characterization.



Fig 3.2 Mass production of EPNs in artificial media



## 4. Turmeric



### Genetic resources

In *Curcuma*, 1270 accessions are being maintained. Wild germplasm exploration and collection were carried out in the Thekkady forests, Idukki district, Kerala. *Curcuma longa* and *C. aromatica* were collected and conserved at the *ex situ* gene bank at IISR, Peruvannamuzhi. The collection is characterized by moderate yield (fresh clump weight-1.23kg), dry recovery-17.19%, moisture-8.19%, oil-3.2%, curcumin-5.42% and oleoresin-13.78% (Fig 4.1).



Fig 4.1 *Curcuma longa* collected from Thekkadi forests

### Evaluation of nematode tolerant turmeric accessions

The experiment conducted through 2009-12 consisted of seven accessions and released variety IISR Prathibha, as check. Various yield and yield contributing characters *viz.*, plant height, tillers/plant, leaves/plant, leaf length, leaf breadth and yield were recorded. Maximum mean yield over three years of 14.15 and 14.11 kg/3m<sup>2</sup> was recorded in Acc. 48 and Acc. 79 followed by IISR Prathibha with 12.93 kg/3m<sup>2</sup>.

### Promising seedling progenies

About 35 promising progenies shortlisted based on earlier trials were planted in replicated trial in the field with released varieties Prabha and Kedaram as controls. In general, the yield was low in treatments as well as control compared to the last year. 16 progenies produced yield above 7.5

kg/bed. 11 progenies showed significantly higher yield compared to the controls. Three progenies yielded above 10 kg/bed. When analyzed for curcumin, oil and oleoresin contents, 13 seedling progenies showed curcumin content above 4%. Seedling progeny 389/1 showed above 5% curcumin consistently during the last three years.

### Chromosome number analysis

Chromosome number was analyzed in 60 seedling progenies. All of them showed deviation from normal chromosome number of 2n=63. Most frequently occurring number was 2n=84. Of the two mother plants analyzed, one showed 2n=63 and other 2n=84.

### Genetic purity testing

Twenty samples were received from one of the planters to test whether they are pure seed material of variety- Prathibha. The samples were profiled with four ISSR primers. The results showed that of the 20 lines tested only five were similar to Prathibha.

### Essential oil profiles

Dried rhizomes of *Curcuma longa* (var. suranjana), *C. amada*, *C. aromatica* and *C. caesia* yielded 3.1%, 1.0%, 3.3% and 1.7% essential oil respectively. The chief components of the oil are as follows : *C. longa*- turmerone (30.6%), ar-turmerone (5.08%) and curlone (15.03%), *C. amada* - myrcene (37.2%), and  $\beta$ -pinene (9.03%), *C. aromatica* - curdione (13.38%), camphor (9.38%) , 1,8- cineole (6.81%), borneol (4.85%), germacrone (3.93%), and camphene (3.07%  $\beta$ -elemene (3.3%) curzerene (4%), neocurdione (4%); and *C. caesia* – curzerenone (17.91%) and 1,8- cineole (9.26%), camphor (2.9%), bornyl acetate (3.6%),  $\alpha$ - terpineol (2.56%), curzerene (5.2%),  $\beta$ -elemene (4.64%).

Essential oil content in 13 varieties *viz.*, Sugandham, Roma, Suroma, Pant Peethabh, Renga, CoI, BSRI, Rajendra sonia ,Varna, Suranjana, Resmi and ranged from 3.1-5.7%.





Highest essential oil content was recorded in Resmi (5.7%) followed by Suroma and Renga with 5%. The major constituents of the essential oil were turmerone (22.6-45.1%), curlone (6.8-21.7%),  $\alpha$ - phellandrene (0.5-5.9%), 1,8- cineol (0.2-2.2%), terpinolene (0.4-5.1%), ar-curcumene (0.9-4.1%), zingiberene (1.4-10.3%), and  $\beta$ -sesquiphellandrene (0.7-10.4%). Turmerone content was maximum in Sugandham (45.1%), which was followed by Roma (40.9%). Resmi contained 31.9% turmerone and 21.7% curlone.

### Cloning of *pal* gene

PCR conditions were optimized using *pal* gene specific primers, designed based on plant transcript assembly database at TIGR. PCR amplified products of 1336, 1335 bp length were obtained using templates of variety Alleppey Supreme rhizome DNA (Fig 4.2). These were cloned into PTZ57R/T and sequenced. Blast analysis revealed sequence identity of up to 97% with *pal* sequences of *Zea mays*, *Musa balbisiana*, *Oryza sativa* Japonica Group and *Salvia miltiorrhiza*.

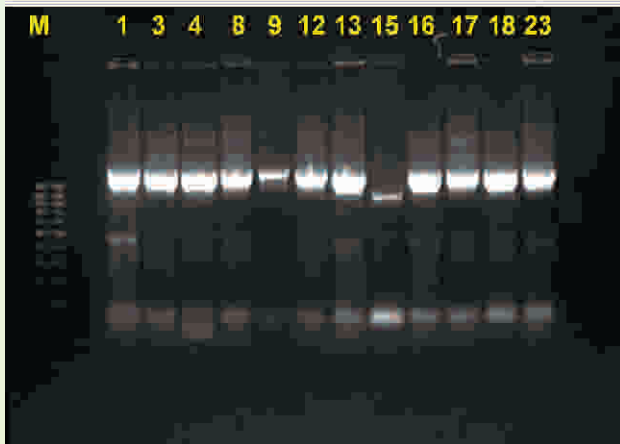


Fig. 4.2 Clones of *pal* Primer TA1.33F/TA1.33R in PTZ57R/T (M: Mass ruler; 1-15: PTZ57R/T positive clones)

### Organic farming

Turmeric was grown organically by applying FYM, vermicompost, ash and rock phosphate, *Azospirillum* and phosphobacteria and *Trichoderma* & *Pseudomonas* sp. (IISR-6) The rhizome yield was higher under integrated systems followed by organic and chemical systems. Application of Panchagavya or Biodynamic culture (BD 501) along with FYM or

with Vermicompost yielded on par with that of integrated management. The enzyme activities was found to be higher under organic and integrated systems. Among organic manures, addition of panchagavya, neem cake and vermicompost along with FYM recorded higher levels of enzyme activities. Significantly higher oleoresin and curcumin contents were recorded under organic on par with integrated management. Under organic management Alleppey Supreme recorded higher oil and starch (50%) contents where as Prathibha recorded higher curcumin (5.6%).

### Economic optimum for nutrient response

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 ₹ 0.65/ bed for N, ₹ 0.40/ bed for P and ₹ 0.85/bed for K.

### Micronutrients on yield and quality

The effect of Zn and B on the quality var. Prathiba was studied. The pooled analysis of three years yield data showed an increased response with soil application of Zn up to 10 kg/ha which tend to decrease at a higher dose of 15 kg/ha (Fig 4.3). The response was more pronounced in the absence of P fertilizer (-P) application. With the application of P fertilizer (+P), significantly higher yield increase was observed up to 5 kg Zn/ha over control and the yield was on par among the higher doses. Application of one or two foliar spays of ZnSO<sub>4</sub> (0.25%) also recorded on par yield with that of soil Zn application. Foliar spray of Zn twice @ 0.25% and soil application @ 10 kg/ha recorded higher curcumin (4.9-6.2) and oleoresin (11.2 – 13.5) contents. Similarly, application of B @ 1 kg/ha without lime application increased the rhizome yield up to 15% compared to control. But when the lime was applied, similar increase in yield was observed under no B supplementation, indicating the benefits of correcting the soil pH for increasing the B availability. Foliar spray of B once or twice @ 0.2% recorded significantly highest yield of 14.8 and 13.7 kg/ 3m<sup>2</sup>, respectively and also increased the curcumin content significantly



(4.86 – 6.08). When applied in combination, application of foliar sprays of B and Zn (once and twice) along with liming or P fertilizers, respectively, recorded higher rhizome yield.

### Shoot Borer

Leaf cuticle wax was estimated in mature leaves of six resistant (Accs. 422,435,589,687,954 and 1026) and three susceptible (Accs. 924, 925 and 1007) accessions. The wax content in susceptible and resistant accessions of turmeric ranged from 0.0058 to 0.0086 and 0.0055 to 0.3311 mg per 200 cm<sup>2</sup>, respectively.

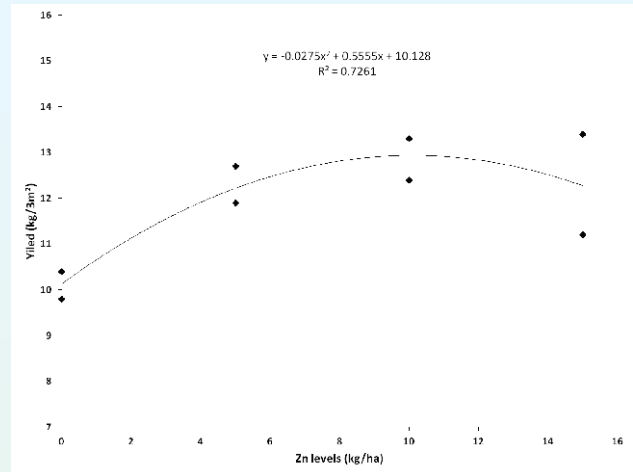
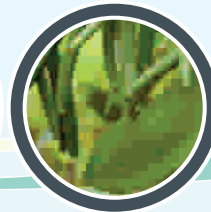


Fig. 4.3 Response of yield in relation to Zn levels



## 5. Vanilla



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

A total of 633 cultures of different interspecific hybrids and seedling progenies from selfing were maintained *in vitro*. 100 interspecific hybrids involving *V. tahitensis* × *V. planifolia* and 100 selfed seedlings of *V. tahitensis* were established *ex vitro*.

### Screening for disease resistance

Ten plants each derived from irradiated protocorms with 0.5 kr and 10kr gamma rays and interspecific hybrids involving *V. planifolia* × *V. tahitensis* were inoculated with *Fusarium oxysporum*. After three rounds of inoculation one

plant derived from the protocorms treated with 0.5kr was found to be free of infection. Others showed varied levels of infection and plant death. Later, mild infection was noticed in plant initially found free of infection.

### Morphological and cytological analysis of interspecific hybrids

Morphological characters like leaf length, leaf breadth and internode length was recorded from 19 interspecific hybrids planted in pots. Chromosome number analysis of two interspecific hybrids between *V. planifolia* and *V. tahitensis* showed  $2n=30$  in one (PT-5) and  $2n=32$  in the other (PT-17).





## 6. Tree Spices



### NUTMEG

Seedlings of a unique nutmeg type with more of hermaphrodite flowers have been collected from Sri Poornanand V Bhat, an innovative farmer from Aversa, Ankola (Uttar Kannada) (Fig 6.1).



**Fig.6.1 Bisexual nutmeg seedlings collected and conserved**

In the trial on clonal evaluation of high yielding lines, A9/185 was found to have significantly more height (720cm), canopy (620cm), girth (65.2cm) and number of primary branches per plant (84) after four years of planting. In the trial on clonal evaluation of lines having high myristicin and elemicin, A9/20 recorded significantly more height (46cm), canopy (56.8cm), girth (1.37cm) and number of primary branches per plant (3), two years after planting. In the trial on clonal evaluation of lines having low myristicin and elemicin and high sabinene, A9/69 recorded more plant height (86.3cm), canopy (56.5cm) and girth (1.45cm) and the maximum number of primary branches per plant (11) after two years of planting.

### Suitability for high density planting

An experiment was laid out at varying spacings viz., 3.0 m x 2.0 m, 3.0 m x 4.0 m, 4.5 m x 4.0 m, 4.5 m x 4.0 m and control to study the suitability for high density planting. About 90% of the plants have established. Morphological characters like

plant height, number of branches etc. were recorded. Plant height ranged from 52-60 cm and number of branches from 2.8 to 4 in various treatments.

### GARCINIA

#### Digitization of *Garcinia* herbarium

All type collections (about 100) of herbarium available at National Herbarium, Kolkata was rephotographed into digital photos. One copy was deposited in IISR library for reference.

Leaf and fruit samples of four species viz., *G. livingstonei*, *G. dulcis*, *G. siamensis*, *G. spicata* and *G. cornea* were collected from National Botanic Garden, Kolkata (Fig. 6.2)



**Fig. 6.2 *Garcinia* specimens collected from national botanic garden**

Molecular profiling of eight species (total 60 accessions) was done with 20 ISSR markers which gave good polymorphic bands. Two bands of 450bp and 700bp are present uniformly in all the species with five different markers.

Chromatographic fingerprinting and estimation of organic acids was done by paper chromatography. Total number of acids varied within 5-7. Total acids was highest in *G. cowa* (Kuji thekara) while lowest was in



*G. mangostina*. Estimation of organic acids using RP-HPLC showed that next to hydroxycitric acid, malic acid is present in high concentration. Other acids present are oxalic acid, citric acid, tartaric acid, acetic acid and ascorbic acid. Dried fruit rinds extraction of eight species namely *G. gummi-gutta*, *G. tinctoria*, *G. indica*, *G. mangostana* and *G. subelliptica* of Western Ghats and *G. pedunculata* (Bor thekera), *G. lancifolia* (Rupohi thekera) and *G. kidya* (Kuji thekera) of

NE Himalayas were analysed for acid profiling and 5-7 organic acids were detected.

### **Garcinia butter**

Hot water extraction and solvent extraction (Methanol/chloroform -1:1) of *G. gummigutta* and *G. tinctoria* seeds yielded 50% butter with yellow colour and pleasant aroma (Fig 6.3). The butter had saponification value of 189.9, 1.42% free fatty acid and cholesterol (0.5%)



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

### Flavour quality and anti oxidant property

#### Black pepper

The sample powdered in pin mill was exposed to 50°C and stored for ten days and evaluated for oil, oleoresin, piperine, total phenol and essential oil constituents. The Powder was stored at 50° C for 10 days both in open and packed condition. Essential oil showed a reduction of 22% in packed and 50% in open condition. Oleoresin showed 13% reduction in packed and open condition and piperine content did not show any significant change. Low boiling constituents like pinene, sabinene, myrcene and limonene were affected. High boiling constituents such as caryophyllene did not show any change. The anti oxidant property was examined using alcohol extract, water extract and petroleum ether extract. Three tests *viz.*, DPPH radical scavenging assay, phospho molybdenum assay and ferric reducing power were carried out for antioxidant test. The pepper samples did not show change in medicinal property

The sample was ground in hammer mill and pin mill and stored for 12 months under modified atmosphere packing (MAP) and ordinary atmosphere at ambient temperature. Reduction in oil was about 34% in both samples after 12 months. About 10-15% reduction was observed in oleoresin, but piperine content did not show any significant reduction. MAP did not show any advantage over other packing with respect to oil, oleoresin and piperine.

#### Turmeric

The var. Prathiba sample powdered in pin mill was exposed to 50°C and stored for ten days and evaluated for oil, oleoresin, curcumin, total phenol and essential oil constituents. Medicinal value of each lot was analysed for examining the anti oxidant property. The samples did not show change in medicinal property.

#### Cinnamon

The bark was ground in pin mill and exposed to 50°C and kept in incubator for 10days both in packed and open condition. The sample was

drawn daily and analysed for oil, oleoresin and total phenol. Essential oil showed reduction of about 25% in packed and 38% in open condition, after a storage period of 10 days. Reduction in oleoresin was negligible. Total phenol content did not show any change. GC-MS profile of oil constituents of bark powder exposed to 50°C for 10 days was recorded. Major constituents like trans-cinnamaldehyde, eugenol, cinnamyl acetate, benzyl benzoate etc did not show significant variation due to exposure to high temperature. Changes have been observed in benzaldehyde,  $\alpha$  &  $\beta$  -phellandrene and phenyl propionaldehyde. The anti oxidant property was examined using alcohol extract, water extract and petroleum ether extract. Three tests namely DPPH radical scavenging assay, phospho molybdenum assay and ferric reducing power were carried out for antioxidant test. The cinnamon samples did not show change in medicinal property

#### Curing of turmeric

Studies on curing of var. Prathiba were conducted in TNAU model steam boiler and by water boiling method. Initial moisture content of rhizomes was 79 per cent and dried to 10 percent after various initial pretreatment like water boiling, steam boiling, slicing and dipping in boiling water. Washed rhizomes was loaded in to the boiler and steamed for varying time intervals *viz.*, 30, 45, 60 and 90 min. Another batch was cooked in boiling water for 40, 60, 90 min. One batch was dipped in boiling water for 10 min. and dried and the last batch was sliced to 3 mm thick and dried. The rhizomes were dried in open cemented yard. The dried rhizomes were tested for their biochemical qualities and the results indicated that slicing reduced the drying time significantly (8 days). Rhizomes cured in improved boiler for 30, 45, 60, 90 min took 18, 16, 11 and 10 days for drying and traditional water boiling for 40, 60, 90 min took 10 days for drying. The reduction in curcumin, starch essential oil, oleoresin and drying time with increased curing time was highly significant by both methods.





### Production of food extrudates from selected spices

Ten flour blends of cassava flour mixed with spice power in the ratio of 96: 4 were prepared and conditioned at 4°C for 15 days. The spices used for blending were mace, turmeric, clove, cinnamon, white pepper, nutmeg, *Curcuma amada*, red chilli, cardamom and black pepper. The blends of cassava flour blended with cardamom powder and the cassava flour blended with pepper powder had good overall acceptability scores of 6.3 and 5.9 respectively compared to the other blends. Extrudates of cassava cardamom and cassava black pepper had the expansion ratios of 2.838 and 3.173, respectively which were high in comparison with the extrudates from the other blends. The bulk densities were low for the extrudates from these blends with a value of 0.158 and 0.14 g/mm<sup>3</sup>. The cassava cardamom extrudate had the least value for hardness (16.346 N) followed by cassava pepper extrudate (20.749 N) thus resulting in good textural properties.

### Mycotoxin contamination

Essential oils of turmeric leaf and *Cinnamomum cassia* bark were tested at concentrations ranging from 0.01% to 1.5 % and 0.01% to 0.5% respectively. Complete inhibition of the

production of aflatoxins was seen at 1.5% (v/v) for turmeric leaf oil, with a drastic reduction in the aflatoxin content from 163 ppb, at 0.75% of the oil to 4.3 ppb at 1.0%. The optimal protective dosage of 1.5% turmeric leaf oil *in vitro* stands good in terms of its practical utility. In the case of *Cinnamomum cassia* bark oil, complete inhibition of the fungal growth was observed at 0.5%. Even at concentration 0.01%, the total aflatoxin production was reduced by 95%.

### Studies on the nutraceutical properties of bioactive compounds in a few spices

Four cancer cell lines – HeLa (human cervical carcinoma cells), MDA-MB-231 (human breast carcinoma cells), HepG2 (human hepatocellular carcinoma cells) and A375 (human melanoma cell line) – were treated with essential oil of black pepper, ginger, turmeric, cinnamon, and curry leaves at 0.01% and 0.02% concentrations, water and ethanol extracts of black pepper, ginger, turmeric, cinnamon, *Garcinia indica*, *G. gummi-gutta*, tamarind and curry leaves at 25 and 50 µg/mL concentrations, for 48 h.

Cancer cells treated with essential oil of the spices - ginger, turmeric, cinnamon and curry leaf (black pepper was less effective) – showed significant decrease in cell viability. Turmeric, cinnamon and curry leaf reduced the viability of HeLa cells

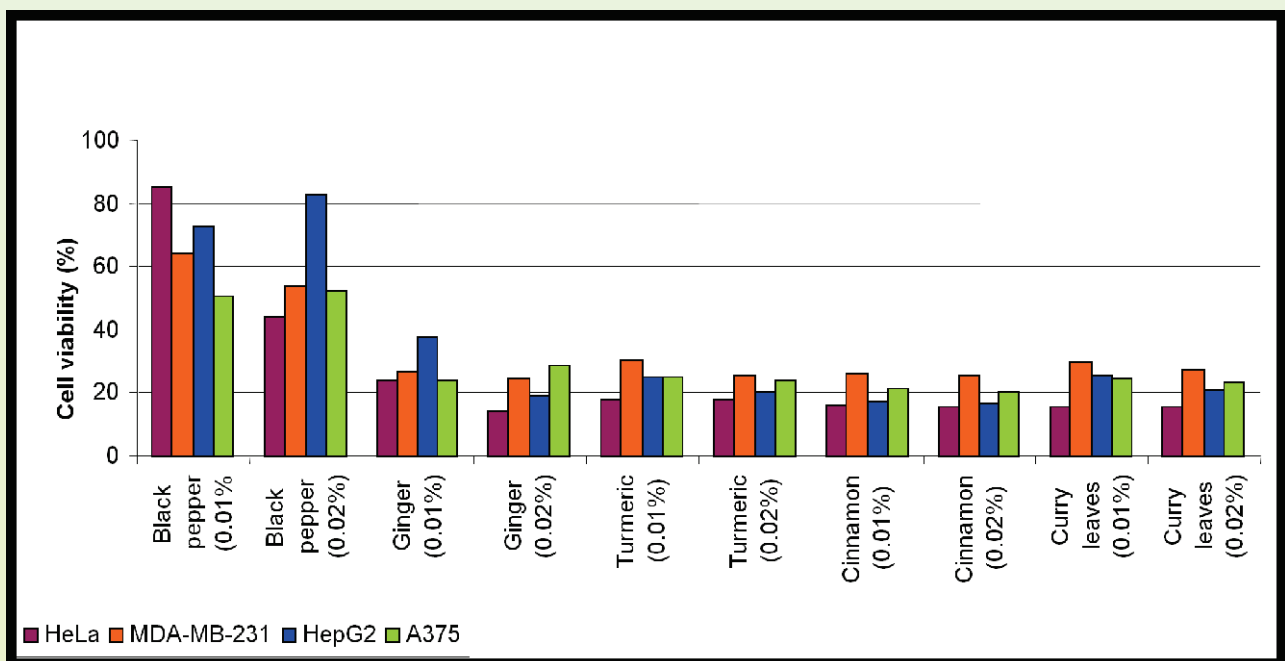


Fig 7.1. Cell viability of four cancer cells treated with essential oil of spices



down to 15-18%, irrespective of concentration; whereas in ginger the cell viability was reduced by half when the concentration of essential oil was doubled from 0.01% to 0.02%. The decrease in viability of breast cancer cells, MDA-MB-231, was on par (24% to 30%) in all spices tested, except black pepper, at both concentrations. The cell viability of HepG2 was affected the most by essential oils of cinnamon and ginger (at 0.02%, double that at 0.01%); the rest were on par, black pepper being least effective. In A375 cells too, black pepper was only one half as effective as the other spices in its cytotoxicity.

Among the water and ethanol extracts, the most effective treatments were the ethanol extracts of turmeric (27% cell viability) and *G. indica* (37%) at 50 µg/ml. The other promising spice treatments

were turmeric water extract (62%), and the ethanol extracts of curry leaf (75%) and *G. indica* (77%), all at 50 µg/ml. The cell viability of MDA-MB-231 cells was most affected by *G. indica*, followed by curry leaf and tamarind, turmeric and cinnamon (Fig 7.1). The treatments that reduced the cell viability of MDA-MB-231 cells by about half (50-53%) were: *G. gummi-gutta* water extract (50 µg/ml), ethanol extract (25 and 50 µg/ml); *G. indica* water extract (50 µg/ml) and ethanol (both 25 and 50 µg/ml). In HepG2 cells too, the cell viability was most affected by *G. indica* (47%) and curry leaf (38%) ethanol extracts, both at 50 µg/ml. The skin cancer cell lines, A375, were most affected by ethanol extracts of *G. indica* (36%) and turmeric (49%), both at 50 µg/ml.



## 8. EXTENSION AND IMPACT ASSESSMENT

### Out reach extension

Two courses on production management of ginger and turmeric were organized, one for a farmers club from SAS Nagar, Punjab during April 2011 and another for a Seed Cooperative Society from Raipur, Chattisgarh during July 2011. Twenty two trainees attended the courses. Exposure training programmes were organized for new field officer and scientists recruits of Spices Board (37 trainees) and for a group of Field extension officers of Department of Horticulture, Kodagu district, Karnataka. Two officers from Jayanthi Spices, a private organisation in Coimbatore also were provided two day training on Post Harvest technology in spices. The institute participated in 2 exhibitions/farmers fares at National level and 7 at regional or state level.

### Technology mission for pepper in Wayanad

About 1500 soil samples from black pepper growing panchayats of Wayanad district were analysed for major, secondary and micro nutrients and results with site specific recommendations was passed on to the farmers. 15% of the soil samples analysed was found to be highly acidic. In available P content 62% of samples analysed were found to have > 40 kg/ha and among which >35% of soils had >100 kg/ha P, which is very high.

About 150 samples were collected from disease hot spot areas in three panchayats and analysed for microbial/ pathogen/ nematode loads were analysed and farmers were advised with management strategy. 76% of samples from diseased gardens and 32% from healthy gardens showed the presence of *Phytophthora*. Ten visits were made by team of scientists to hot spot/ problem areas along with KAU scientists and advisories were given to the farmers. Pamphlets were prepared in Malayalam on composting, use of pesticides, biocontrol of pest and diseases and distributed to farmers.

### Multi enterprise farming models to address the agrarian crisis of Wayanad District of Kerala

During the past year 9000 cuttings of black pepper of varieties of IISR, Kozhikode were supplied to RARS, Ambalavayal. Varieties supplied are

Malabar Excel, Panchami, Sreekara, Girimunda, Thevam, Pournami, Shakthi. Training on IPM and IDM in major spices held at Wayanad Social Services Society, Mananthavady on 26<sup>th</sup> May 2011. Impact assessment on the 5000 black pepper cuttings distributed at Mananthavady during 2009-2010 was made. The study indicated 83% survival/ establishment.

### Soil based plant nutrient management plans for agro-ecosystems of Kerala

The project envisages analyses of 17,069 soil samples of 87 Panchayats of Calicut district, analyses for pH, OC, major-, secondary- and micro-nutrients, uploading the data into [www.keralasoilfertility.net](http://www.keralasoilfertility.net). The results revealed that the mean soil pH across the 22 Panchayats was 5.5 and mean SOC level was  $1.1 \pm 0.54\%$ . About 26% of the soil samples were low in SOC (0.4 -0.7%). Mean available P level was  $114.35 \pm 83 \text{ kg ha}^{-1}$  and 9% of the samples had high (25-35  $\text{kg ha}^{-1}$ ), 30% had very high (36-100  $\text{kg ha}^{-1}$ ) and 25% of the soils recorded extremely high (> 100  $\text{kg ha}^{-1}$ ) available P. Mean exchangeable K level was  $251 \pm 176 \text{ kg ha}^{-1}$  and a major percentage of the samples (55%) were medium (116 - 275  $\text{kg ha}^{-1}$ ) in exch. K, Mean exchangeable Ca level was  $522 \pm 394 \text{ kg ha}^{-1}$  with 60% of the samples recording adequate levels (>300  $\text{kg ha}^{-1}$ ) and 32% exhibiting low levels (151- 300  $\text{kg ha}^{-1}$ ). Mean exchangeable Mg level was  $90 \pm 78 \text{ kg ha}^{-1}$  and only 19% of the samples were adequate in exch. Mg (> 120  $\text{kg ha}^{-1}$ ) and a major percentage of the samples were inadequate in exch. Mg (37% low and 44% very low). Mean available Zn level was  $5 \pm 8.5 \text{ ppm}$  and majority of the soils (91%) exhibited adequate Zn level (> 1.0 ppm), In case of available Cu, 96% of the samples were adequate (> 1.0 ppm). Mean B level across the 22 Panchayats was  $1.0 \pm 1.8 \text{ ppm}$  with 26% of the samples registering deficiency in B (< 0.50 ppm). However, a vast majority of the samples (49%) was adequate (> 0.50- 2.0 ppm). Mean available S level was  $10 \pm 22 \text{ ppm}$  and 26% of the sample was adequate and 33% of the samples were low (< 5ppm) in available S. Nutrient advisories have been generated for 22 Panchayats.





### Media visits

Three media visits were arranged to various demonstration units of KVK and IISR experimental farm and progressive farmers fields. Eight journalists from various english/malayalam newspapers/farm magazines and radio channels participated in the visits. More than 40 Success Stories and 130 news items (Coverage of Kisan Mela, Success Stories, Media Visits, New Varieties, Technologies, etc) were published.

### Audio/video programmes

Five radio programmes and four TV news clippings were produced and 15 audio capsules were broadcasted through AIR, Calicut. Special programmes on IISR and spices cultivation were broadcasted in Mattoli FM and AIR Kannur FM.

Four documentary films were produced on,

- Rewriting the fate of Queen of Spices: A Success Story from Kodagu
- George Panackavayal: Harvester of Hope
- Cassava - The Bread of Tropics: Ensuring livelihood to poor farmers
- *Kalpavriksha* brings happiness in their homes

### Kisan mela and technology showcasing

'Krishi Jalakom 2012' & Technology Expo were organized from February 16-18, 2012 at IISR

Campus. Mr. Sparjan Kumar, IPS, District Police Chief Inaugurated the farmers meet, Dr. M. Anandaraj, Director, IISR presided and Dr. M. Tamil Selven, Director, DARD, Kozhikode opened the exhibition stalls. Twenty organizations including public and private displayed their technologies and products in the exhibition. Over 400 farmers attended the meet and more than 1000 people visited the stalls.

Turmeric farmer Mr. Chandrasekhar Azad, Andhra Pradesh, Dairy Farmer Mr. John Joseph, Kodanchery, Black Pepper farmer Mr. George, Vazhapparambil and remote operated coconut climbing machine developer Mr. Prakash, Kozhikode were felicitated on the occasion.

### Awareness programme on PPV&FR Act 2001

An Awareness programme on the "Provisions of Protection of Plant Varieties and Farmers Rights (PPV&FR) Act 2001" Sponsored by PPV&FRA, New Delhi was organized at institute on Friday, 17 February 2012. Smt. R Ushamani, Principal Agricultural officer, Department of Agriculture, Calicut, inaugurated the programme and Dr. S Ramachandran, Director, Regional Science Centre and Planetarium, Kozhikode presided over the function (Fig 8.1). One hundred and forty registered farmers participated actively in the awareness programme. Agricultural Officers and Scientists from different institutions also participated in the function.



Fig. 8.1. PPV & FR programme: Inaugural function



## 9. ALL INDIA COORDINATED RESEARCH PROJECT ON SPICES

The All India Coordinated Research Project on Spices (AICRPS) is located in Kerala with its headquarters at IISR, Kozhikode. The independent AICRPS which formed with 14 centres in 12 states has grown to 34 centres spread over 21 states of the country representing various agroclimatic zones suitable for spices. The mandate crops include black pepper (9 centres) small cardamom (4 centres) fennel (9 centres) fenugreek (12 centres) Tree spices viz., clove, cumin and nutmeg (3 centres each). The XI plan (2007-2012) budget of AICRPS is ₹ 1637.50 lakhs with ₹ 656.5 lakhs (ICAR share) for 2011-12.

### Black pepper

The germplasm of black pepper collected and maintained at 7 AICRP centers is about 750 accessions. Sirsi centre has identified two promising high yielding lines viz., Ademane pepper (Acc. 53) with field tolerance to phytophthora and Kudragutta which possess field tolerance to drought. Two comparative varietal trails involving promising lines of black pepper are in progress at different centers. Culture 5489 is most promising at Panniyur, while Culture 5308 and HP 1411 were best at Pampadumpara. Culture 5308 performed best at Chintapalle also followed by Panniyur -1. Studies on rooting of orthotropics shoots in black pepper revealed that two nodal cuttings with IBA 1000 ppm dip gave the highest percentage (85%) of rooting at Yercaud but at Peechiparai two nodal cuttings with half the concentration of IBA (500 ppm) gave the best (80%) result. Trials at Sirsi reported 100% rooting when 5 nodded cuttings are treated with IBA 400 ppm. Trials at Panniyur indicated integrated nutrient management (INM) gave maximum green berry yield when compared with either fully organic or fully inorganic fertilizers were used. Black pepper vines applied with new molecules of fungal toxicants @ 0.1% Fenamidon 10% + Mancozeb (50%) (Sectin) as spray @ 2 lit/vine or 3 lit/vine as drench along with bio agent *Trichoderma harzianum* (MTCC-5179) 50 g with one kg of neem cake as soil application two times

- first during first week of June and another during third week of August is effective in controlling *Phytophthora* foot rot in Karnataka and Andhra Pradesh.

### Cardamom

The germplasms collected and maintained at 2 AICRP centers is about 322 accessions. The promising cardamom clones CL-722, CL-726 and CL-691 identified from Mudigere were under coordinated varietal trials. Acc. S-1 (CRSP-160), a long panicle malabar type and Acc. PS-27 (CRSP-80), an oleoresin rich, bold and greenish capsule type is promising at Pampadumpara. In the CVT 2009 series accessions DL-14 IC-34 987 were promising with respect to yield attributes. Combined application of bacterial consortium (IISR-6 and IISR-859) and *Trichoderma harzianum* (MTCC-5179) each @ 25 g/plant resulted in effective management of tiller, panicle and capsule infection by *Phytophthora meadi* at Pampadumpara centre.

### Large cardamom

Five new accessions viz., SCC-227 (Ramsey) SCC 228 (Varlangey) SCC-229 (Ramsey) SCC-230 (Ramla) SCC-231 (Golsey) were collected and added to germplasm. In addition 14 blight (caused by *Colletotrichum gloeosporioides*) escapes were collected from hot spot areas of Sikkim and Dargeeling hills of West Bengal and are under multiplication at ICRI, Gangtok.

### Ginger

About 534 accessions were maintained at 6 AICRP centers. Studies on Genotype × Environment interaction on ginger revealed local check SG-827 followed by Himigiri at Solan, Varada followed by Suprabha at Pundibari, and GCP-5 at Chintapalle with maximum yield. Biofumigation using mustard as well as cabbage waste resulted in the lowest incidence of soft rot and bacterial wilt in ginger disease at Pundibari, Kumarganj and Solan centres. Trials at Pundibari and Raigarh indicated integrated nutrient management (INM) gave maximum yield in



ginger when compared with either fully organic or fully inorganic fertilizers were used. However inorganic nutrient treatment produced highest yield at Pundibari.

### Turmeric

About 1372 accessions were maintained at 10 AICRP centers. Studies on Genotype  $\times$  Environment interaction on turmeric revealed, Narendra Haldi followed by BSR-2 performed best at Kumarganj, TCP-2 followed by Narendra Haldi-1, IISR Kedaram at Pundibari, Roma at Chintapalle and Suranjana at Raigarh centres with highest yield. Application of FYM 5 t/ha + inorganic N (50%) + Azospirillum 5 kg/ha soil application has given higher yield of 41.66 t/ha in turmeric with a BC ratio of 3.23 compared to control (20.16 t/ha) at Coimbatore. Recommended dose of NPK + FYM + application of consortium of *T. viride* & *Pseudomonas fluorescens* @ 4 g/kg and 12.5 kg/ha respectively (seed and soil) was effective for the management rhizome rot in turmeric at Coimbatore centre. Rhizome treatment with carbendazim + mancozeb (1:1) and spray (1%) after 45 and 40 DAP is recommended by Raigarh centre for foliar disease management in turmeric. At Chintapalle, rhizome treatment with Propiconazole (0.1%) + foliar spraying on 45 and 90 DAP recorded lowest incidence of leaf spot and with respect to leaf blotch, rhizome treatment with carbendazim (0.1%) on 45 to 90 DAP resulted in lowest incidence. It was found that rhizome treatment as well as foliar spraying with Hexaconazole (0.1%) at 45 and 90 days after planting is the best treatment in controlling both leaf blotch and leaf spot disease at Pundibari centre.

### Tree spices

About 122 nutmeg, 53 cinnamon and 39 clove germplasm were maintained and evaluated at the centers. Among 24 clove germplasm accessions SA-13 recorded the highest yield (3.89 kg/ha). In the CVT of clove SA-3 gave the highest dry bud yield of 2.90 kg/tree. In nutmeg, accession MF-4 recorded the maximum fruit yield of 999 fruits/tree. Nutmeg A/150 gave good performance in the CVT at Peechiparai. Out of 12 cinnamon accessions, Sel. 65 was found promising with high dry bark yield (645 gm/tree) at Peechiparai. Keeriparai -1 with one meter length

and 5-6 cm thickness recorded the maximum dry weight of quills 130 g and 828 quillings and featherings as well as highest essential oil (2.6%) and oleoresin (9.1%) in bark oil at Peechiparai.

### Coriander

About 2128 accessions of coriander were maintained at various AICRP centers. Mutation breeding was initiated and elite genotypes for earliness, bushy nature, leaf and dual purpose were identified in coriander. Drought tolerant lines viz., LCC-143, LCC-159, LCC-164, LCC-165, LCC-200 at LCC – 183 were identified from germplasm at Guntur centre. Coriander acc. RCr-684 followed by UD-476 were relatively stable and high yielding in the drought trials across the year at Jobner. Studies on effect of rhizobacteria on growth and yield of coriander indicated that maximum seed yield was obtained with the seed and soil application of rhizobacteria FL-18 followed by Trichoderma (MTCC-5179 at Hisar. Encouraging results has been obtained with the use of Rhizobacteria FK -14 and FL-18 in yield and growth promotion at Raigarh. However the effect of different PGPR bioformulations on growth and yield characters and yield were non significant at Jagudan.

Technology for Coriander leaf production during summer months was also standardized at Guntur, which offers better net return to farmers. The entries LCC-232, LCC-242 and LCC-244 identified as suitable for summer leaf production. Similar technology for off season (summer) production of leafy coriander was also standardized at TNAU. Cultivation of variety CS-11 for leafy purpose under 50% shade has been successful with a leaf yield of 6.71 t/ha as against the cultivation under open conditions (3.76 t/ha). The benefit cost ratio was also high 5.80 as against cultivation under open conditions. Nutrient management in offseason leaf production, nutrients and growth regulators treatment increased the leaf production in coriander during off season. Soil application of 45:40:20 NPK + combined with spray GA 15 ppm 20 days after sowing recorded maximum growth and yield parameter. At Hisar among the three shaded levels 50% shade resulted in higher yield followed by 75% shade. Among the entries tested DH-228 produced maximum leaf yield followed by DH-259. Application of Tricentanol (1 ml/lit of water)





as foliar spray (at 40, 60 and 80 days) after sowing is effective in increasing the seed yield at Kumarganj and Coimbatore centres. Irrigation using rain gun in coriander has been identified as the best method for increased water use efficiency and high returns at Guntur. In nutrient supplementation through organic manure maximum yield obtained by application of recommended INM (2086 kg/ha) followed by application of 100% nitrogen supplemented with vermi compost @ 5 t/ha and RDF at Hisar.

Seed treatment and drenching with Calixin (0.1%) + spray with Calixin (0.1%) after 60 DAS has recommended for the control of powdery mildew and stem gall at Jobner. Seed treatment and soil drench with Tridemorph (0.01%) + spray with Tridemorph (0.01%) at 60 DAS and spraying with wettable sulphur (0.2%) at 60 DAS recommended by Jagudan. Seed treatment with *Pseudomonas fluorescens* (IISR-6) @ 10 g/kg of seed followed by foliar application at  $10^8$  cfu on 60 day after sowing effectively reduced the powdery mildew at Coimbatore. Minimum stem gall disease in recorded when seeds are treated with Hexaconazole (@ 0.20%) + spray at 45, 60 and 75 DAP which were at par with seed treatment and spray with propiconazole @ 0.20% at Raigarh.

### Cumin

About 590 accessions were maintained at various AICRP centers. Acc. UC 239, UC -274 and UD-225 were the stable genotypes in the limited moisture conditions of Jobner. Studies on yellowing and sugary disease (Gummosis) in cumin conducted at Jobner revealed that no biotic factors are involved in the initiation of disease and sugary disease is a physiological disorder which is due to prolonged low temperature, high humidity and frequent irrigation at the time of

flowering. Application of *Trichoderma harzianum* @10 kg/ha + FYM @ 3 t/ha recommended the minimum disease incidence of 1.78% and maximum seed yield at Jobner in the management of biocontrol agents. Beneficial effect of PGPR bioformulation by application of rhizobacteria FL-18 and FK-14 as seed and soil treatment were found promising in increasing the yield at Jobner and Jagudan centres.

### Fennel

About 651 accessions were maintained at various AICRP centers. Fennel variety RF-145 has been identified for National release from Rajasthan. Maximum seed yield obtained with application of *Trichoderma* MTCC-5179 followed by rhizobacteria FL-14 seed treatment at Hisar. The effect was not significant due to seed pelleting of both PGPR formulations individually as well as in combination at Gujarat. Studies on the efficiency of different insecticides and botanicals on seed wasp in fennel at Jagudan revealed that thiamethoxam 25 WG @ 0.0084% sprays at three and seven days after harvest had recorded the least damage (8.73%).

### Fenugreek

About 1118 accessions were maintained at various AICRP centers. Genotypes UM-11 followed by UM-24 were found to be most drought tolerant lines at Jobner while Accs. UM-10, UM-8, UM-13, UM-26 and RMt-1 were stable genotypes in both irrigated and drought conditions. Two foliar sprays of 50 ppm NAA at 40 and 60 DAS resulted in higher seed yield as well as net return for fenugreek at Jobner. Significant beneficial effect of PGPR bio formulations FK-14 + FL-18 were obtained in fenugreek at Jagudan.



## 10. BIOINFORMATICS

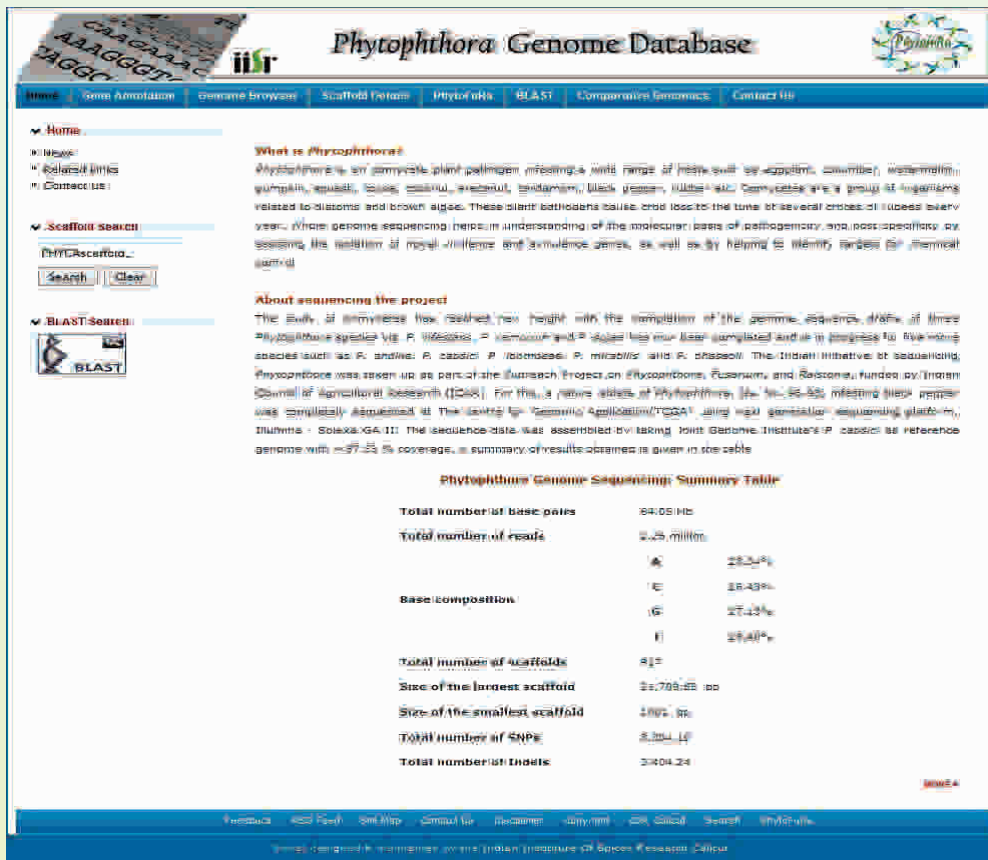
### Phytophthora genomics

The whole genome sequence data of a native isolate of *Phytophthora capsici* (Is. No. 98-93) infecting black pepper was assembled by taking Joint Genome Institute's *P. capsici* as reference genome. Functional annotation of the whole genome sequence of *P. capsici* infesting black pepper is in progress.

*In silico* studies on protein-protein interaction in *Phytophthora*-black pepper interaction was conducted. Computational analysis of signal peptide dependent effector proteins in the plant pathogen *P. capsici* have been carried out. Functional annotation of SNPs in *P. capsici* was carried out and deleterious nsSNPs were identified through *in silico* analysis.

### New databases developed

A new database, *Phytophthora* Genome Database (<http://220.227.138.212/genomedb/>) based on *Phytophthora* whole genome sequencing and annotation was developed (Fig 10.1). The database provides access to primary structure of the *Phytophthora* genome including genome sequence, number of genes, CDS, SNPs, inDels, nucleotide composition, intron-exon structure, start and stop codon, intron lengths, alternative splicing and untranslated regions (UTRs) to the research community. GenomeView, a next-generation stand-alone genome browser and editor developed at Broad Institute is used as the genome browser (Picture). Another database on *Radopholus* genus called RADOBASE (<http://www.spices.res.in/radobase>) was developed and launched. This database contains comprehensive information on sequence and morphological details of 22 *Radopholus* species.



**Phytophthora Genome Database**

Home | Gene Annotation | Genome Browser | Scaffold Filter | Phytozink | BLAST | Comparative Genomics | Contact Us

**What is Phytophthora?**  
Phytophthora is an oomycete plant pathogen resembling with fungi of which over 100 species, including watermelon gummy rot, squash, black rot, bacterial, and blight, black rot, and blight. Phytophthora is a group of organisms related to diatoms and brown algae. These plant pathogens cause and lose to the tune of several crores of rupees every year. Whole genome sequencing heralds understanding of the molecular basis of pathogenicity, and possibly offers an avenue for the isolation of novel virulence and avirulence genes, as well as by helping to identify targets for chemical control.

**About sequencing the project**  
The bulk of this project has reached new heights with the completion of the genome sequence of one of three *Phytophthora* species viz. *P. infestans*, *P. ramorum* and *P. capsici* has now been completed and is in progress for five more species such as *P. andreae*, *P. capsici*, *P. lateralis*, *P. microbotrytis* and *P. obovata*. The Indian initiative of sequencing *Phytophthora* was taken up as part of the Outreach Project on Phytophthora, Fusarium, and Botrytis funded by Indian Council of Agricultural Research (ICAR). For this, a series of workshops on *Phytophthora* (in 2005-06) meeting their needs was completely supported at The Centre for Genome Annotation (CGA) and high quality sequencing platform, Illumina - Solexa-GA II. The sequence data was assembled by taking Joint Genome Institute's *P. capsici* as reference genome with >97-98% coverage. A summary of results obtained is given in the table.

Total number of base pairs	84.05 Mb	
Total number of reads	2.5 million	
Base composition	A	28.3%
	T	28.43%
	G	27.13%
	C	28.40%
Total number of scaffolds	812	
Size of the largest scaffold	21,708,628 bp	
Size of the smallest scaffold	1892 bp	
Total number of SNPs	3,304,167	
Total number of indels	33,04,224	

Fig 10.1 *Phytophthora* genome database



## 11. AGRICULTURAL KNOWLEDGE MANAGEMENT UNIT

AKMU facilitates the maintenance of the Local Area Network (LAN) of the Institute and ensures uninterrupted net connectivity to all divisions/sections. The repair and maintenance of computers, printers and accessories of various divisions/sections is facilitated through AKMU. The Personnel Management Information System Network – II (PERMISnet II) and Project Information & Management System of ICAR (PIMS-ICAR) data were updated by AKMU. Apart from this, AKMU assists in statistical analysis of scientific data using SAS and other

statistical software.

During the year the institute was connected to the pan India ultra high bandwidth network called National Knowledge Network through 100 Mbps optical fibre connectivity. The existing office automation software, ARISoft has been modified into a fully web based software with additional features and functionality. A new module for e-procurement has been developed during the period and integrated with ARISoft (Fig 11.1 a&b).



(a)



(b)

Fig 11.1 (a) Home page of e-procurement module (b) Home page of ARISoft





## 12. LIBRARY

The library website was revamped with links to additional digital resources and databases. The library continued to be a consortium partner of CeRA, the e-journal consortium of ICAR. It subscribed the CAB Direct online and 20 foreign journals and 67 Indian journals. Spice Bibliography, a searchable online database was compiled with records starting from the year 1910. A new bibliography service, PhytoLib was conceived, developed and launched. Twelve issues of the online news service 'AGRI titbits' were published. A class on "Science direct online; key strategies for using the e-resource" was conducted by Shri. Ravisankar (Elsevier). A workshop cum training programme on CeRA was

conducted by Dr. A.K. Mishra, Scientist (SS) & Co-PI (CeRA), IARI, New Delhi. The scope of the digital institutional repository, DSpice was widened with more institute publications. In connection with digitization of books 32 important books were scanned and hosted in the open source software greenstone. Online access to library resources was extended to CRC, Appangala and the IISR Experimental Farm, Peruvannamuzhi. The library procured 138 books, 13 technical reports, six theses and five project reports on *gratis* base during the period. Around 205 external users and 2750 internal users made use of the library facilities.



Fig 12.1 Home page of SpicE-Library

## 13. AGRICULTURAL TECHNOLOGY INFORMATION CENTRE

The ATIC is involved in technology dissemination functions through a single window, coordinating with various divisions of IISR. The major activities are

- Sale of planting material produced at main campus
- Sale Bio control agents
- Sale of institute publications and extension literature
- Diagnostic services including soil, plant and manure sample testing
- Advisory/Information services to farmers through visits, letters, telephone and e-mail
- Development of audio visual aids

### Technology inputs

The technology inputs distributed from the centre include quality planting material of improved varieties of spices, bio control agents and scientific publications including extension literature. During the year planting materials worth ₹ 182290 was distributed. The sale registered an increase of 11.3% over the previous

year. The proceeds from sale of publications amounted to ₹ 18770 indicating a four fold decrease compared to previous year. *Trichoderma* and *Pseudomonas* formulations worth ₹ 147995 were distributed to farmers. The sale of bio agents showed a phenomenal 15 times increase over previous year. The total income generated was ₹ 356155.

### Farmer visit and advisory services

During the year, 897 farmers (365 from within the District, 220 from the state and 312 from outside state) availed farm advisory services from the centre. Nine hundred and twenty two students visited the institute for study purpose. Eleven groups of farmers (one from Tamil nadu, four from Karanataka, two from Kerala, one each from AP, Maharashtra, Rajasthan and Pondicherry) visited under sponsored study tour programmes. The total visit recorded was 1882. From the secondary data recorded on the pattern of information seeking behavior of farmers showed: Direct visits – 1882; Phone calls – 544; Letters – 142 and e mail – 144.



## 14. KRISHI VIGYAN KENDRA

KVK has conducted 162 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 6324 trainees were benefited out of the programmes.

### Friends of coconut

Friends of coconut is a training programme aimed at providing employment opportunities for the unemployed youth and was conducted in collaboration with Coconut Development Board, Cochin. A total of 159 youth were trained under this programme. One training was conducted exclusively for women. After the training, six trained women out of 25 trainees, selected coconut climbing as their profession. The women can climb up to 30-50 trees per day, earning a daily income of ₹ 600/-. Before the training, male climbers got ₹ 200-300 per day. They could climb a maximum of 20- 30 trees per day. But after training with the help of the machine, the trained climbers are earning ₹ 700 per day through coconut climbing since they can climb 70 coconut palms per day.

### FLD programmes

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

- Demonstration of HYVs of black pepper with high intrinsic qualities
- Demonstration of bush pepper production technology using Karimunda variety
- Integrated disease management of *Phytophthora* foot rot of black pepper
- Demonstration of pheromone traps for control of fruit fly in bittergourd.
- High density planting of tissue culture nendran banana
- Demonstration of serpentine method of multiplication of planting material in black pepper.
- Demonstration of vermicomposting using poultry manure
- Composting of poultry carcass
- Cage culture of pearl spot (*Etroplus suratensis*) in brackish water area
- Cage culture of *Pangasius* sp. (Tiger shark) in large fresh water areas

### Technology assessment and refinement

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

- Performance evaluation of mixed cropping of nutmeg variety *Viswasree* graft in coconut gardens.
- Induction of flowering in *Olour* mango through paclobutrazol application combined with INM and IPM
- Integrated nutrient management in choice variety of cassava based on soil test data for higher yield
- Protected cultivation of vegetables using low cost rain shelter.
- Use of human placenta extract to augment fertility in repeat breeder crossbred cows.
- Effect of probiotic supplementation on growth performance in heifer calves
- Use of biofilters and probiotics in maintaining water quality in ornamental fish culture tanks.
- Quality analysis of nutmeg products





### Revolving fund programme

The kendra has a strong revolving fund programme to generate income and under this programme, quality planting materials of various crops are produced and made available to public at affordable rates. Income also was generated by way of sale of layer chicks, goats, heifers and bulls and consultation and doorstep services through the clinic, sale of fingerlings, bioproducts, biocontrol agents etc. During the period, an amount of ₹ 11.78 lakh has been realised through sale of various technological inputs to farmers.

### 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	- 682
Artificial insemination	- 285
Animal health campaigns/infertility camps	- 11
Vaccination of poultry birds and animals	- 2572
Block <i>ksheeroltsavam</i>	- 4

### KVK website

A website ([www.kvkcalicut.gov.in](http://www.kvkcalicut.gov.in)) for KVK was launched in Malayalam for the benefit of farming community of Calicut. The site contains information about all the mandatory activities of KVK, success stories, planting material availability and links to market information in addition to online registration for training programmes.

### Kisan mobile advisory service through SMS

KVK has begun 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 24 SMS benefitting 742 farmers and 100 extension functionaries.

### Soil testing campaigns and issue of soil health cards

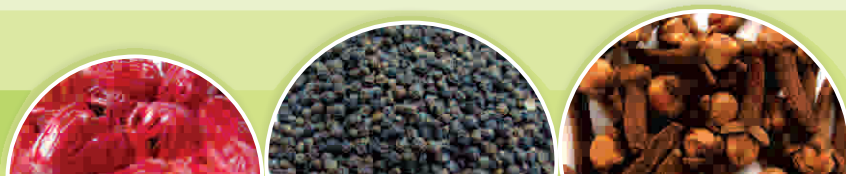
Soil testing campaigns were conducted in all the four wards of Chakkittapara panchayat and soil health cards were issued to sixty farmers. A total of 14 soil health campaigns were conducted so far and altogether 1275 soil samples were analysed.

### Farmers' field school (FFS)

A farmers' field school on Breeding and culture of ornamental fishes was conducted at Thamarassery, Quilandy, and Nochad panchayats involving 25 farmers. Training programmes, method demonstrations, field visits, farmers' group meetings were organised as part of the programme. Inputs for ornamental fish culture were also provided to participatory farmers.

### Other extension activities

KVK conducted three seminars, participated in five Kisan Mela cum exhibitions, broadcasted seven radio talks and conducted one study tour for farmers to various research institutions during the period as detailed below. Folder were published on Broiler goat rearing, Soil testing for reducing cost and increasing yield and Caring of pregnant cows. Booklets were published on Nutrition garden, Anthurium cultivation and Banana cultivation.



Sl. No.	Activity	Date	No. of participants
<b>Seminar</b>			
1	Micro ornamental fish culture for JLG clusters	19 November 11	138
2	Spices Production Technology	28-29 December 2011	100
3	Spices Production Technology	10-11 January 2012	100
<b>Exhibition</b>			
1	Haritholsavam 2011, Wholesale Agri. Market, Maradu, Cochin	3-7 September 2011	1000's
2	Haritholsavam 2011, College of Agriculture, Vellayani, Trivandrum	19-24 September 2011	1000's
3	Suvarnadarsanam 2011, Govt. Higher Secondary School, Quilandy	1-3 December 2011	1000's
4	Karshika Mela 2012, Thodupuzha	27 December 2011 to 1 January 2012	1000's
5	Kisan Mela & Technology Week (Krishi Jalakam) 2012, IISR, Calicut	16-18 February 2012	1000's
<b>Farmers' study tour</b>			
1	Freshwater fish culture farm, Koovapoyil	9 January 2012	34

### Participatory seed production in ginger and turmeric

In order to enhance the production of quality planting materials, the farmer participatory seed production programme taken up during last year was continued in ginger and turmeric. Under the programme, KVK has identified five potential

turmeric farmers and five ginger farmers in Calicut and monitored at field level for scientific seed production. Good quality seed material produced was procured at KVK and sold to needy farmers. A total of 400 kg turmeric (IISR Prabha) and 800 kg of ginger (IISR Varada) were sold to 120 farmers.



## 15. RESEARCH PUBLICATIONS

1. Ankegowda, S.J, Venugopal, M.N., Krishnamurthy, K.S. and Anandaraj, M. 2011. Impact of basin irrigation on black pepper production in coffee based cropping system in Kodagu District, Karnataka. *Indian J. Hort.* 68: 71-74
2. Aravind, R., Kumar, A., Dinu, A. and Eapen, S.J. 2011. Single tube duplex PCR for simultaneous detection of *Phytophthora capsici* and *Radopholus similis* infecting black pepper (*Piper nigrum*). *Indian Phytopath.* 64: 353-357.
3. Bhadramurthy, V, Bhat, A.I. and Biju, C.N. 2011. Occurrence and coat protein sequence based characterization of *Bean yellow mosaic virus* (BYMV) associated with vanilla (*Vanilla planifolia* Andrews) in India. *Archives Phytopathol and Plant Protection* 44: 1796-1801.
4. Elizabeth Thomas, Zachariah, T.J., Syamkumar, S. and Sasikumar, B. 2011. Curcuminoid profiling of Indian turmeric. *J. Medicinal Aromatic Pl. Sci.* 33: 36-40.
5. Dhanya, K., Syamkumar, S., Sijusen and Sasikumar, B. 2011. SCAR markers for adulteration detection in ground chilli. *British Food J.* 113: 656-668.
6. Dhanya, K., Syamkumar, S., Sijusen and Sasikumar, B. 2011. Sequence characterized amplified region markers: A reliable tool for adulterant detection in turmeric powder. *Food Res. International.* 44: 2889-2895.
7. Dinesh, R., Anandaraj, M., Srinivasan, V. and Hamza, S. 2012. Engineered nanoparticles in the soil and their potential implications to microbial activity. *Geoderma.* 173-174: 19-27.
8. Dinesh, R., Srinivasan, V., Hamza, S., Manjusha, A. and Sanjay Kumar, P. 2012. Short-term effects of nutrient management regimes on biochemical and microbial properties in soils under rainfed ginger (*Zingiber officinale* Rosc.). *Geoderma*, 173-174: 192-198.
9. El-Sharkawy I, W El-Kayal, D Prasath, Fernandez H, Bouzayen M, Svircev AM, S Jayasankar (2012) Identification and genetic characterization of a gibberellin 2-oxidase gene that controls the tree stature and reproductive growth in plum. *J. Exptl. Bot.* 63: 1225-1239.
10. Jaleel Kizhakkayil and Sasikumar, B. 2012. Characterisation of ginger (*Z. officinale* R.) germplasm based on volatile and non volatile components. *Afr. J. Biotech.* 11: 777-786.
11. Jayashree, E. and Visvanathan, R. 2011. Physical and biochemical parameters of fresh and dry ginger (*Zingiber officinale* Roscoe). *J. Spices and Aromatic Crops.* 20: 14-21.
12. Jayashree, E. and Visvanathan R. 2011. Studies on mechanical and thermal properties of fresh and dry ginger. *J. Spices and Aromatic Crops.* 20 : 60-65.
13. Jiby, M.V. Bhat, A.I. 2011. An Efficient *Agrobacterium*-Mediated Transformation Protocol for Black Pepper (*Piper nigrum* L.) Using Embryogenic Mass as Explant. *J. Crop Sci. Biotech.* 14 : 247-254.
14. Kizhakkayil, J. and Sasikumar, B. 2011. Diversity, characterization and utilization of ginger: A review. *Plant Gen. Resources C&U.* 9: 464-477.
15. Krishnamurthy, K.S., Kandiannan, K., Sibin, C., Chempakam, B. and Ankegowda, S.J. 2011. Trends in climate and productivity and the relationship between climatic variables and productivity in black pepper. *Indian J. Agric. Sci.* 81: 729-733.
16. Kumar, A., Prameela, T.P., Suseela Bhai, R., Siljo, A., Biju, C.N., Anandaraj, M. and Vinatzer, B.A. 2011. Small cardamom (*Elettaria cardamomum* Maton.) and ginger (*Zingiber officinale* Roxb) bacterial wilt is caused by same strain of *Ralstonia solanacearum*: a result revealed by multilocus sequence typing (MLST). *European J. Plant Pathol.* 132: 477 – 482.
17. Maheswarappa, H.P., Srinivasan, V. and Lal, R. 2011. Carbon footprint and sustainability of agricultural production systems in India. *J. Crop Imp.* 25: 303-322.
18. Parthasarathy, V.A., Srinivasan, V., Nair, R.R., Zacharia, T.J., Kumar, A. and Prasath, D. 2011. Ginger: Botany and horticulture. *Hort. Review.* 35: 273-388.
19. Prasath, D., El-Sharkawy, I., Sherif, S., Tiwary, K.S. and Jayasankar, S. 2011. Cloning and characterization of PR5 gene from *Curcuma amada* and *Zingiber officinale* in response to





- Ralstonia solanacearum* infection. *Plant Cell Reports*. 30: 1799–1809.
20. Rethesh, S.T. and Bhat, A.I. 2011. Genetic transformation and regeneration of transgenic plants from protocorm-like bodies of vanilla (*Vanilla planifolia* Andrews) using *Agrobacterium tumefaciens*. *J. Plant Biochem. Biotechnol.* 20: 262–269.
  21. Rosana O.B., Dinsha M., Shamina A. and Santhosh J.E. 2012. Virtual screening and in vitro assay of potential drug like inhibitors from spices against Glutathione-S-Transferase of *Meloidogyne incognita*. *Bioinformation*. 8: 319-325.
  22. Santhosh J. Eapen, Ramana, K.V. and Saji, K.V. 2011. Host resistance in black pepper (*Piper nigrum* L.) against root knot and burrowing nematode. *J. Plantation Crops*. 39(3): 341-346.
  23. Sasikumar, B. 2011. Biodiversity in Ramayana, *AsianAgri-History*. 15:247-249.
  24. Shamina, A., Rosana, O.B., Riju, A. And Reena, N. 2012. Virtual screening and in vitro assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes. *J. Molecular Modeling*. 18: 151-163.
  25. Siljo, A., Bhat, A.I., Biju, C.N. and Venugopal, M.N. 2012. Occurrence of *Banana bract mosaic virus* on cardamom. *Phytoparasitica*. 40:77-85.
  26. Simi Mohan, Utpala Parthasarathy and K. Nirmal Babu. 2012. In vitro and in vivo adventitious bud differentiation from mature seeds of three *Garcinia* spp. *Indian J. Natural Products and Resources*. 3 : 65-72.
  27. Sindhu, S., Chempakam, B., Leela, N.K. and Suseela Bhai, R. 2011. Chemoprevention by essential oil of turmeric leaves (*Curcuma longa* L.) on the growth of *Aspergillus flavus* and aflatoxin production. *Food Chem. Toxicol.* 49: 1188-1102.
  28. Srinivasan, V., Maheswarappa, H.P. and Lal, R. 2012. Long term effects of topsoil depth and amendments on particulate and non particulate carbon fractions in a Miamian soil of Central Ohio. *Soil & Tillage Res.* 121: 10–17.
  29. Thankamani, C.K. and Ashokan, P.K. 2011. Effect of drip irrigation on yield, nutrient uptake of bush pepper (*Piper nigrum* L.) inter cropped in coconut garden. *J. Medicinal Aromatic Plant Sci.* 33: 365-368.
  30. Thankamani, C.K., Kandiannan, K., Madan, M.S., Raju, V.K., Hamza, S. and Krishnamurthy, K.S. 2011. Crop diversification in black pepper gardens with tuber and fodder crops. *J. Plantation Crops*. 39 : 358-362.



## 16. EDUCATION AND TRAINING

### National training programme on Allele mining

A national training programme on 'Allele Mining' sponsored by National Agricultural Innovation Project, was organized at this institute from September 12-25, 2011. The national training, exposed the trainees to the use of genomic technologies along with genetic and bioinformatics approaches for identifying allelic variations and to dissect trait-gene associations. The programme covered theoretical and practical sessions in the areas of molecular markers and diversity analysis, development of core collections using morphological descriptors and molecular markers, motif- directed profiling and cloning, full length gene cloning, next generation sequencing technologies, tools for allele mining, promoter mining, functional genomics, bioinformatics tools for gene annotations and allele mining, etc.

Fourteen trainees from crop science, horticulture, natural resource management, fisheries, animal science background participated in the programme. Dr. M Abdul Salam, Vice Chancellor, University of Calicut, inaugurated the fourteen day training programme. Dr. Johnson George K was the Course Director and Dr. Santhosh J Eapen and Dr. D Prasath served as course coordinators. Dr. HP Singh, Deputy Director General (Horticulture), Indian Council of Agricultural Research (ICAR), New Delhi was the chief guest on the valedictory function (Fig 16.1).

### Training on whole genome sequencing

A DBT sponsored training program on "Whole genome sequencing and annotation" was conducted during February 21- 25, 2012 with 14 participants from various research institutions and universities. The contents included were next generation sequencing, sequence assembly and visualization, annotation and variant analysis, transcriptome analysis and comparative genomics.

### Post Graduate studies

#### Ph.D

Mr. PS. Manoj, Horticulture, Simulation of variability and induction of growth in mangosteen (*Garcinia mangostena*), KAU, Thrissur.

Ms. Sindhu, Biochemistry, Biochemical investigations and post harvest management of aflatoxin contamination in black pepper, ginger and turmeric, Mangalore University.

### MSc/ Post MSc training

Eight M.Sc./M.Tech students carried out project work in various disciplines.

### Summer training for MSc students

One month summer training on Biochemistry, biotechnology and bioinformatics was conducted for 14 M.Sc. students during 3<sup>rd</sup> May – 4<sup>th</sup> June 2011



Fig 16.1 DDG (Horticulture), ICAR, presiding over the valedictory function



### Trainings attended by the staff

Officials attended	Training programme	Date	Organization
Dr. Utpala Parthasarathy, Technical Officer	Training on Data mining and GIS for decision support in agriculture	28 March to 8 April 2011	IIM, Lucknow
Dr. K. Nirmal Babu, Principal Scientist	Capacity building programme on Holistic foundations for assessment and regulation of genetic engineering and genetically modified organisms	2-7 May 2011	Centre for Sustainable Agriculture, Hyderabad
Dr. T. K. Jacob, Principal Scientist	Short course on high performance bio-computing and drug design	12-23 September 2011	IASRI, New Delhi
Mr. V.V. Sayed Mohammed and Mr. P. Sundaran, Assistants	Training on Financial decision making using excel	26-30 September 2011	National Institute of Financial Management, Faridabad
Dr. S.J. Ankegowda, Senior Scientist	National training on allele mining	10-21 October 2011	TNAU, Coimbatore
Dr. T.K. Jacob, Principal Scientist	Training on DNA barcoding of insect pests of horticultural crops	7-11 November 2011	IIHR, Bangalore
Dr. N.K. Leela, Senior Scientist	DST sponsored training on Innovation management and technology management for scientists	7-18 November 2011	Administrative Staff College of India, Hyderabad
Dr. C.K. Thankamani and Dr. J. Rema, Principal Scientists	DST sponsored training on General management programme for middle & senior level women scientists	21 November – 2 December 2011	Administrative Staff College of India, Hyderabad
Dr. S. Hamza, Technical Officer	DST sponsored training on Innovation management & technology valorization	12–23 December, 2011	Administrative Staff College of India, Hyderabad
Dr. R. Praveena, Scientist	Training on Effectiveness enhancement programme for scientist	16-20 January 2012	Institute of Management Training and Research, Goa.





Mrs. Shyna Deepesh and Ms. Chinchu E K, Stenographers	Hindi computer training programme	13-17 February 2012	National Power Training Institute, Bengaluru
Mr. K.G. Jegadeesan, AFACO and Mr. V.V. Sayed Mohammed, Assistant	Programme on financial systems, management and accountability in government	13-17 February 2012	ASCI, Hyderabad
Dr. S.J. Anke Gowda, Senior Scientist	Phenotyping and molecular breeding for improving drought adaptive traits in crops	13-22 February 2012	University of Agricultural Sciences, Bangalore.
Dr. Rashid Pervez, Senior Scientist	Training for Hindi officers	19-23 March 2012	Central Hindi Training Institute, New Delhi

### International deputations

Officials attended	Training programme	Date	Organization
Dr. R. Senthil Kumar	NAIP training on marker assisted selection	15 March-14 June 2011	Iowa State University, Iowa, USA
Dr. A.I. Bhat	NAIP training on Biosecurity (Hort.)	28 March-27 June 2011	Food and Environment Research Agency (FERA), Sand Hutton, York, UK,
Dr. T. John Zachariah	Institute of Food Technologists Annual Meeting & Food Expo-2011	11-14 June 2011	New Orleans, Louisiana, USA
Dr. K. Nirmal Babu	2 <sup>nd</sup> International symposium on under utilised plant species – crops for the future	27 June to 1 July 2011	University of Nottingham, Malaysia
Dr. Utpala Parthasarathy	2 <sup>nd</sup> International symposium on under utilised plant species – crops for the future	27 June to 2 July 2011	University of Nottingham, Malaysia
Dr. T.E. Sheeja	NAIP training on marker assisted selection	18 July- 16 October 2011	College of Agriculture and Life Sciences, Cornell University, Ithaca, USA
Dr. M. Anandaraj	Second Asian PGPR conference	21-24 August 2011	Beijing, China



## 17. INSTITUTE TECHNOLOGY MANAGEMENT UNIT

Regular meetings of ITMU were conducted for the effective review and implementation of issues related to commercialization of technologies and IP protection. In 2011-12, nine license agreements have been signed with six farmers for the commercial multiplication of three turmeric varieties and one ginger variety. Total revenue of 2.48 lakh was generated from the initiative. Another agreement for the commercial production and sale of *Trichoderma harzianum* was signed with Pest control India Pvt Ltd. The patent application for “The process of production of high quality white pepper and in particular production of off-odour (foul) free white pepper with less microbial load using bacterial fermentation technology” was filed through the patent attorney (The Patent office journal 20/04/2012 pp. 6182). ITMU conducted awareness programmes on Intellectual Property rights & Plant variety registration in workshop/seminar organised for biology teachers and farming community respectively.

The applications of software databases viz., Spice genes, SPICEST, Spice Prop, PLASBID, PASSCOM, Phytoweb, Juzbox were sent for copyright registration. The ITMU participated in various technology promotion activities including the Horticulture-Industry Meet/Convention at IIHR, at Bangalore during 5-6 March 2012 and International Conference-cum-Exhibition “Food 360<sup>0</sup>”, at Hyderabad during 20-22, November 2011 (Fig 17.1).



Fig 17.1 Participation at the International Conference-cum- Exhibition “Food 360<sup>0</sup>”

### Technologies for commercialization

#### Nutrient mixtures for spices

This is a novel soil pH based micronutrient mixture for promoting growth, yield and quality of turmeric, ginger & cardamom. Under proper conditions it can be stored for up to one year/ one crop season. It is recommended as foliar spray at the rate of 5 g/litre on 60<sup>th</sup> and 90<sup>th</sup> day after planting in case of turmeric and ginger and as foliar spray at the rate of 5 g/litre in May-June and September-October every year in case of black pepper and cardamom. An approximate increase of up to 15% in yield and a cost benefit ratio of 1:2.5 is expected (Fig 17.2).



Fig 17.2 Nutrient mix for spices

#### A Simple and easy PGPR technology for ginger:

This PGPR formulation enhances nutrient mobilization and nutrient use efficiency, growth and yield and provide protection against diseases at a negligible cost. It can be applied to rhizomes prior to planting. Booster doses of the same PGPR can be given as soil drench.

#### Microbial consortium for enhanced growth and yield in black pepper:

It can be applied both in black pepper nurseries and under field condition as soil drench or along with FYM. Roots when dipped in microbial formulation improves rooting and performance of plants (Fig 17.3).

The products were showcased during the Horticulture Industry Meet held at IIHR, Bengaluru on 06 March 2012. Efforts are on for issuing non-exclusive licenses to these technologies.



Fig 17.3 PGPR mix for spices



## 18. HINDI CELL ACTIVITIES

The Official Language Implementation Committee (OLIC) meets once in every quarter and reviews the official language implementation activities of the Institute. Quarterly and annual reports on official language activities of the institute has been prepared and sent to ICAR, New Delhi, TOLIC, Kozhikode and Regional Implementation Office, Cochin. The half yearly report on Official Language implementation also has been prepared and submitted to Regional Implementation Office, Cochin.

Four workshops were organized at IISR, Kozhikode. First; Use of computer in official language implementation on 17<sup>th</sup> June 2011, second; Noting and drafting on 20<sup>th</sup> September 2011, third; How to popularize OL in the office on 22<sup>nd</sup> December 2011 and forth; Use of computer for the implementation OL in the office on 7<sup>th</sup> March 2012. Aaj ka Shabd in Hindi with its transliteration in English is being displayed daily on the board. Hindi Day was celebrated on 14<sup>th</sup> September 2011 and Hindi Week on 14-21<sup>st</sup> September 2011. During this week various

competitions viz., extempore speech, song, debate, noting and drafting, memory test, caption writing, Samasya Pooran and anthakshari were conducted for the staff members and prizes were distributed to the winners in the valedictory function on 21<sup>st</sup> September 2011. Dr. R. Surendran, Former Head, Department of Hindi, Calicut University was the Chief Guest (Fig 18.1).

Dr. Rashid Pervez, Senior Scientist & Hindi Officer and Ms. N. Prasannakumari, Hindi translator attended subcommittee meeting of TOLIC at SBT, Kozhikode on 12<sup>th</sup> August 2011. Dr. Rashid Pervez and Ms. N. Prasannakumari attended the 48<sup>th</sup> TOLIC meeting on 17<sup>th</sup> October 2011 at Kovilakam Residency, Govindapuram, Near MIMS Hospital, Kozhikode. Dr. Rashid Pervez, attended TOLIC sub committee meeting at SBT, Calicut on 2<sup>nd</sup> February 2012 and valedictory function of Joint Hindi Fortnight of TOLIC on 2<sup>nd</sup> March 2012 at Regional Science Centre, Kozhikode.



Fig. 18.1 Hindi week valedictory function





## 19. RECOGNITION

### Tolic Rajbhasha shield 2011

Institute received TOLIC Official language Implementation Award 2011 from Town Official Language Implementation Committee (TOLIC), Calicut for the implement of the official language activities in the institute like, implementing of

rules, hindi correspondence, conduct of hindi workshops, hindi week and publications such as Masala Samachar (biannually), Anusandhan ke mukhya ansh, extension bulletin i.e. Vanilla, Spicing the nation and popular articles in hindi (Fig 19.1).



Fig 19.1 Tolic Rajbhasha Shield 2011



## 20. INSTITUTE MANAGEMENT COMMITTEE

- |   |                  |
|---|------------------|
| 1. Dr M Anandaraj, Director, IISR, Calicut                      | Chairman         |
| 2. Dr B Raju, Director of Education, UHS, Bagalkot              | Member           |
| 3. Dr R Dhanapal, Head, Crop Production, CPCRI, Kasaragod       | Member           |
| 4. Shri P Balabrahmaiah, SFAO, CPCRI, Kasaragod                 | Member           |
| 5. Shri Sulfikar Mayoore, Kayangulam, Alappuzha                 | Member           |
| 6. Shri CV Damodaran, Kattukulangara, Kanhangad                 | Member           |
| 7. Shri Ashok Macrin, Asst. Director of Horticulture, Nagarcoil | Member           |
| 8. Mr B Krishnamurthy, Pr. Scientist, IISR, Calicut             | Member           |
| 9. Mr V Mohanan, AO, IISR, Calicut                              | Member Secretary |



## 21. RAC RECOMMENDATIONS 2012

S No	Recommendations	Comments of the Director
1.	In order to avoid duplication of germplasm accessions, the IC numbers must be retained by the coordinating centers.	For the collection made by the coordinating centres, IC numbers are obtained by the respective Universities and a set of germplasm is deposited with the National Active Germplasm Centre (NAGS), IISR, Kozhikode. Similarly the collection given to coordinating centres from NAGS is with IC numbers
2.	Holistic systematic programme on germplasm exploration from unexplored areas need to be worked out jointly and collection should be undertaken in a phased manner by all NAGS centers.	Whenever explorations are made to unexplored areas, both NBPGR and the concerned State Agricultural University through AICRPS will be involved as is being done presently.
3.	Varietal spread and performance of nutmeg (IISR – Viswashree) need to be studied and documented.	Addresses of farmers to whom IISR Viswashree has been supplied during the past years are documented. The performance of Viswashree in these places will be recorded.
4.	Unique farmer's varieties of notified spice crops should be registered with PPV & FRA and also unique germplasm shall also be documented and registered.	Any unique varieties of spices, if found with any farmers, will be registered with PPV & FRA.
5.	Under high density cropping studies – pruning response of tree spices should be understood properly for improving efficiency besides standardizing water management.	This item of work would be carried out as a technical programme during the current year onwards.
6.	Nitrate Nitrogen in the soil can be monitored in future as it is of environmental importance.	This can be done in the ongoing fertilizer trials.
7.	Role of weather and soil factors influencing curcumin content may be worked out based on G x E analysis in order to develop GAP for different regions.	The necessary information has already been gathered in the ongoing AICRP trials. Analysis will be done during the current season.
8.	Bioprospecting of spices for assessing their anticancerous properties and identification of indicator molecules.	This work will be taken up under the proposed platform project on high value compounds and phytochemicals.





9.	A viable method for curing turmeric without losing quality may be standardized for different scales of production and processing.	A new experiment will be started during the current year.
10.	Institute should have a separate wing for planting material production which should be managed by technical staff.	Appropriate action will be taken up after the IRC 2012 by the Division of Crop Production and Post Harvest Technology.
11.	In view of several fungi found associated with rhizome/root rot affecting cardamom, efforts may be made to identify the primary causal pathogens.	In the new research project on rhizome/root rot, this work will be done to pin-point the primary pathogens.
12.	While evaluating new biocontrol agents against respective pathogens, the recommended isolates may also be included in the trials for comparative efficiency.	The recommended isolates would be included in the future trials.
13.	Create awareness on viral diseases and virus indexing among all stake holders.	Awareness on viral diseases and virus indexing would be created through training programmes for extension officials/farmers and also through publications
14.	Organize Institute - Industry partnership programmes for commercialization of technologies such as post harvest technologies, machineries, multiplication of bio agents etc.	This would be done during the current year.
15.	Collaborate with other institutes to develop value added products, product diversification and licensing.	Collaboration with appropriate institutes will be sought.
16.	Impact assessment of training programmes and technologies in terms of quantifiable indicators may be studied with help of KVK.	This will be initiated during the current year.
17.	Bee keeping activities may be encouraged to enhance pollination and KVK shall have a programme on bee keeping.	Bee keeping would be encouraged in the mandate areas of KVK to enhance pollination of various crops and also as a source of additional income for the farmers.
18.	Overall outcome of research during XI plan may be compiled and technologies be listed for transfer to farmers.	The outcome of research will be reviewed during the ensuing IRC. Technologies to be transferred would be finalized after reviewing it.



19.	During the XII plan, flagship programmes at Institute level may be finalized and implemented for mandate crops.	Three flagship programmes at the institute level for the XII Plan for various mandate crops would be finalized during the IRC 2012 meeting.
20.	Representatives of line departments, promotional agencies and sister R & D organizations may be invited to RAC meetings for better interaction, exchange of findings and also accelerate the process of technology dissemination.	Representatives of the line departments, promotional agencies and R&D organizations will be invited in future RAC meetings for better interaction.
21.	Experiments on precision farming and protected cultivation shall be initiated.	Experiments on precision farming and protected cultivation in black pepper would be initiated.
22.	Studies on response of important spice crops to climate change or impact of climate change on biology and production of major spices crops may be started in major production areas in collaboration with NICRA project.	A project proposal for getting funding under NICRA would be initiated to study the impact of climate change on production of major spice crops in major areas.
23.	Technologies for mass multiplication and liquid formulations of biocontrol consortia may be developed, which are easy for application.	Trials for developing liquid formulations of biocontrol agents would be initiated.
24.	Nanotechnology, ICT, remote sensing and GIS may be integrated in ongoing and future research programmes for improving research efficiency.	A separate proposal under nanotechnology would be taken up during XII Plan.
25.	Special attention needed for locating sources of resistance for biotic and abiotic stresses using conventional and biotechnological tools for developing varieties with high yield, quality and specific traits.	The programmes on addressing biotic and abiotic stresses are in progress. However, emphasis will be given for locating the sources of resistance.
26.	Knowledge on genomics and bioinformatics need to be strengthened with capacity building.	Capacity building on frontier areas such as genomics and bioinformatics needs to be strengthened by training scientists in the relevant field.



## 22. LIST OF PROJECTS

### I. Institute projects

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

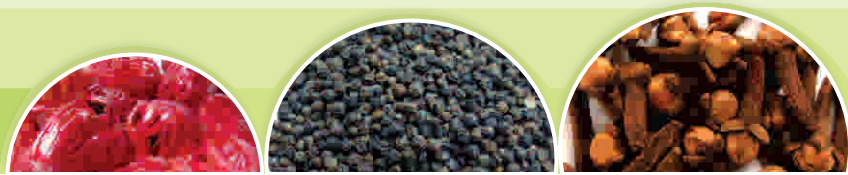
1. Gen. XXVIII 813: Conservation and characterization of *Piper* germplasm (2008-2014) [KV Saji and R Senthil Kumar]
2. Gen. XIX (813): Conservation, characterisation, evaluation and improvement of *Zingiber* and *Curcuma* Spp (2007-2012) [D Prasath, B Sasikumar and KV Saji]
3. Gen. IX (813): Conservation and characterization of cardamom germplasm (2007-2012) [R Senthil Kumar, CN Biju and TR Usha Rani]
4. Gen. XXVI (813): Evolving high yielding and high quality nutmeg clones by selection (2007-2016) [B Krishnamoorthy and J Rema]

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

1. Gen. XVII (813): Breeding black pepper for high yield and caryophyllene (2007-2016) [KV Saji and T John Zachariah]
2. Gen. XVII (813): Breeding black pepper for *Phytophthora* resistance (2007-2016) [K Nirmal Babu, TE Sheeja and Suseela Bhai]
3. Gen. XXI (813): Breeding black pepper for resistance to “pollu” beetle (2007-2016) [KV Saji and S Devasahayam]
4. Gen. XXII (813): Breeding black pepper for tolerance to drought (2007-2016) [TE Sheeja and KS Krishnamurthy]
5. Gen. X (813): Breeding cardamom for high yield and disease resistance (2007-2012) [R Senthil Kumar and R Praveena]
6. Gen. XV (813): Investigations on the reasons and solutions for the absence of seed set in ginger (*Zingiber officinale* Rosc.) (2005-2012) [R Ramakrishnan Nair and D Prasath]
7. Biotech X (813): Development of core ESTs and cloning of genes from *Piper nigrum* and *P. colubrinum* (2008-2012) [Johnson K George and KS Krishnamurthy]
8. Biotech. IX (813): Development of transgenics for resistance to *Phytophthora* and drought in black pepper (2006-2014) [K Nirmal Babu and TE Sheeja]
9. Gen. XXV (813): Genetics of seedling progenies of turmeric (*Curcuma longa* L.) (2007-2013) [R Ramakrishnan Nair]
10. Biotech XI (813): Identification of molecular markers linked to *Katte* resistance genes in small Cardamom (*Elettaria cardamomum* (L.) Maton) (2009-12) [TR Usha Rani, R Senthil Kumar, R Praveena, D Prasath and K Nirmal Babu]
11. Gen. XXX (813): Evaluation of genetic variability in vanilla with emphasis to disease tolerance (2010-2015) [R Ramakrishnan Nair]
12. Gen. XXIX (813): A comparative study of molecular and bio-chemical diversity of *Garcinia* of Eastern Himalayas and Western Ghat ranges with GIS (2008-2013) [Utpala Parthasarathy, K Nirmal Babu and R Senthil Kumar]

#### Mega Project III: System approach for sustainable production of spices [Project Leader: R Dinesh]

1. SSC V(813): Studies on allelopathy in tree species-black pepper interactions (2009-2013) [R Dinesh and S Hamza]
2. Agr. XXVIII (813): Input use efficiency in turmeric in relation to quality (2007-2012) [K Kandiannan and V Srinivasan]





3. SSC VI (813): Nutrient balance and soil C sequestering potential of spice crops under different management systems (2011-2015) [V Srinivasan, R Dinesh, S Hamza and SJ Ankegowda]
4. Agr. XXIX (813): Effect of weed management practices on growth, yield and quality parameters of ginger (2011-2014) [CK Thankamani and K Kandiannan]
5. Hort. VII (813): Evaluation of nutmeg for its suitability for high density planting (2011-16) [J Rema and PA Mathew]

#### **Mega Project IV: Production physiology of spice crops [Project Leader: SJ Ankegowda]**

1. Phy. X (813): Evaluation of black pepper and cardamom elite lines for yield and quality under moisture stress (2010 – 2015) [SJ Ankegowda and KS Krishnamurthy]
2. Biochem VI(813): Influence of biochemical factors on curcuminoid levels in turmeric (2008-2011)[B Chempakam and A Shamina]
3. Phy. XI (813): Source sink relationship, endogenous hormone levels and their relationship with rhizome development in ginger and turmeric (2011-2014) [KS Krishnamurthy and K Kandiannan]

#### **Mega Project V: Value addition and post harvest processing of spices [Project Leader: T John Zachariah]**

1. Biochem VII (813): Management of mycotoxins in black pepper, ginger, turmeric and nutmeg (2008-2012) [B Chempakam and R Suseela Bhai]
2. PHT. V (813): Studies on improved processing and quality evaluation of major spices (2010-2013) [E Jayashree, T John Zachariah and NK Leela]
3. PHT. VI (813): Studies on production of food extrudates from selected spices (2011-14) [E Jayashree, TJ Zachariah and Thajudeen Sheriff (CTCRI)]

#### **Mega Project VI: Propagation studies in spice crops [Project Leader: C K Thankamani]**

1. Hort. V (813): Rootstock intervention to manage root infection of Phytophthora and nematodes in black pepper (2006-13) [PA Mathew]

#### **Mega Project XIII: Investigations on nutraceutical and pharmacokinetic aspects of spices [Project Leader: A Shamina]**

1. Biochem. III (813): Studies on the nutraceutical properties of bioactive compounds in a few spices (2007-2012) [A Shamina and NK Leela]
2. Biochem. V (813): Cloning of pal gene from turmeric (*Curcuma longa* L.) (2008-2012) [A Shamina and TE Sheeja]
3. Org. Chem. III (813): Flavour profiling of *Zingiberaceae* spices (2008-2012) [NK Leela and S Hamza]

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

1. Path. XIX (813): Development of diagnostics for viruses infecting small cardamom (*Elettaria cardamomum* Maton) (2008 – 2012) [CN Biju and A Ishwara Bhat]

#### **Mega Project VIII: Conventional and molecular approaches for developing pest, pathogen and nematode resistance in spice crops [Project Leader: R Suseela Bhai]**

1. Ent. XIII (813): Screening of germplasm accessions of spices and evaluation of antibiosis resistance to major insect pests (2006-2013) [TK Jacob and S Devasahayam]
2. Path. XX (813): Screening of *Piper* germplasm accessions against *Piper yellow mottle virus* (PYMoV) (2008-2012) [A Ishwara Bhat and TK Jacob]



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

1. Crop. Prot. 1.5 (813): Integrated management of *Phytophthora* foot rot and slow decline diseases of black pepper (2008-2014) [R Suseela Bhai, Santhosh J Eapen and Rashid Pervez]
2. Nema. IV (813): Role of phenyl propanoids in black pepper - burrowing nematode interactions (2008-2011) [Santhosh J Eapen and A Shamina]
3. Nema V (813): Survey and identification of efficient entomopathogenic nematodes (EPNs) against major insect pests of ginger and turmeric (2008-2012) [Rashid Pervez, Santhosh J Eapen and S Devasahayam]
4. Path. XXI (813): Diversity of rhizome – root rot pathogens and their antagonists in cardamom. (2010-2014) [R Praveena and CN Biju]

**Mega Project XI: Extension and Training [Project Leader: P Rajeev]**

1. Ext. IV(813) : Training of research and extension personnel (2005-2012) [P Rajeev]
2. Ext V(813): A study on diffusion, adoption and impact of varieties released from IISR and scientific crop management practices (2006-13) [P Rajeev]

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

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

**II. Externally aided projects**

**i) Department of Biotechnology, New Delhi**

1. DBT-CIB-4: Development of microsattelite markers, molecular characterization of small (*Elettaria cardamomum* Maton) & large cardamom, (*Amomum subulatum* Roxb.), identify core collections and developing data base of important genotypes (2006-2012) [K Nirmal Babu, TE Sheeja and R Senthil Kumar]
2. DBT-CP-4: Accredited Test Laboratory (ATL) under the national certification system for tissue culture raised plants (NCS-TCP) (2008-2013) [A Ishwara Bhat and K Nirmal Babu]
3. DBT-CP-3: Genetic transformation of black pepper to confer resistance against viruses (2006-2012) [A Ishwara Bhat and R Suseela Bhai]
4. DBT-SS-1: Distributed information sub-centre (2000-2012) [Santhosh J Eapen]

**ii) Indian Council of Agricultural Research, New Delhi**

1. ICAR-CP-4: Application of microorganisms for agriculture and allied sectors (AMAAS) : Nutrient management, PGPR and biocontrol (2006-2012) [M Anandaraj and R Dinesh and NK Leela]
2. ICAR-CPPHT-1: Network project on organic farming (2007-2012) [V Srinivasan, C K Thankamani and T John Zachariah]
3. ICAR Mega Seed Project: Seed production in agricultural crops and fisheries (2006-2012) [K Kandiannan and PA Mathew]
4. Outreach project on *Phytophthora*, *Fusarium* & *Ralstonia* diseases of horticultural and Field crops (2008-2012) [M Anandaraj, K Nirmal Babu, R Suseela Bhai, Santhosh J Eapen, and Johnson K George]



5. Outreach programme on management of sucking pests in horticultural crops: (2009-2012) [TK Jacob and S Devasahayam]
6. Outreach programme on diagnosis and management of leaf spot Diseases in Field and Horticultural Crops (2009-2012) [CN Biju and R Praveena]

**iii) National Horticultural Mission, New Delhi**

1. NHM-CPPHT-1: Production of nucleus planting materials of improved varieties of spice crops (2005-2012) [CK Thankamani, S Hamza and SJ Ankegowda]

**iv) 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 (2009-2012) [T John Zachariah and NK Leela]
2. NAIP-SS-I: Multi-enterprise farming models to address the agrarian crisis of Wayanad district of Kerala” Under Component-3: (Sustainable Rural Livelihood Security) of NAIP (2008 - 2012) [R Dinesh, CK Thankamani and TK Jacob]
3. NAIP SS-II: Mobilizing mass media support for sharing agro-information (2009-2012) [TJ Zachariah, P Rajeev and TK Jacob]

**v) State Planning Board, Govt. of Kerala**

1. Kerala State-CPPHT-1: Soil based nutrient management plan for agro ecosystems of Kerala (2010-2012) [R Dinesh, V Srinivasan and S Hamza]
2. Kerala State – CPPHT-2: Pepper Rehabilitation Package – Technology Mission on Black pepper for Wayanad – SUGANDHI (2010-2012) [V Srinivasan, TK Jacob, R Suseela Bhai, R Dinesh, CK Thankamani, K Kandiannan, A Ishwara Bhat, Santhosh J Eapen, SJ Ankegowda, Senthil Kumar, KS Krishnamurthy, P Rajeev, C Biju, S Hamza]



## 23. PERSONNEL

### HEADQUARTER

#### Scientific

Sl.No.	Name	Designation
1	Dr. V. A. Parthasarathy	Director – up to 30 June 2011
2	Dr. M. Anandaraj	Director –from 04 July 2011 & Project coordinator (Spices)
3	Dr. S. Devasahayam	Head, Crop Protection Div.
4	Dr. T. John Zachariah	Head, Crop Production & PHT
5	Mr. B. Krishnamoorthy	Principal Scientist (Plant Breeding)
6	Dr. B. Chempakam	Principal Scientist (Biochemistry)
7	Dr. K. Nirmal Babu	Principal Scientist (Plant Breeding)
8	Dr. B. Sasikumar	Principal Scientist (Plant Breeding)
9	Dr. T.K. Jacob	Principal Scientist (Entomology)
10	Dr. J. Rema	Principal Scientist (Horticulture)
11	Dr. Johnson K. George	Principal Scientist (Gen. & Cytogenetics)
12	Dr. C.K. Thankamani	Principal Scientist (Agronomy)
13	Dr. R. Dinesh	Principal Scientist (Soil Science)
14	Dr. R. Ramakrishnan Nair	Sr. Scientist (Gen. & Cytogenetics)
15	Dr. R. Suseela Bhai	Sr. Scientist (Plant Pathology)
16	Dr. K. Kandiannan	Sr. Scientist (Agronomy)
17	Dr. P. Rajeev	Sr. Scientist (Agril. Extension)
18	Dr. K.S. Krishnamurthy	Sr. Scientist (Plant Physiology)
19	Dr. Santhosh J. Eapen	Sr. Scientist (Nematology)
20	Dr. N.K. Leela	Sr. Scientist (Org. Chemistry)
21	Dr. A. Ishwara Bhat	Sr. Scientist (Plant Pathology)
22	Dr. V. Srinivasan	Sr. Scientist (Soil Science)
23	Dr. A. Shamina	Sr. Scientist (Bio chemistry-PS)
24	Dr. K.V. Saji	Sr. Scientist (Economic Botany)
25	Dr. T.E. Sheeja	Sr. Scientist (Biotechnology)
26	Dr. D. Prasath	Sr. Scientist (Horticulture)
27	Dr. Rashid Pervez	Sr. Scientist (Nematology)
28	Dr. E. Jayashree	Scientist, Senior Scale (AS & PE)
29	Ms. N. Krishna Radhika	Scientist (Biotechnology) – up to 13 Oct. 2011
30	Ms. P. Uma Devi	Scientist (Biotechnology) – from 21 Dec. 2011

#### Technical Officers

1	Dr. Johny A. Kallapurackal	Technical Officer (T9)
2	Dr. Hamza Srambikkal	Technical Officer (Lab) (T7-8)
3	Dr. Utpala Parthasarathy	Technical Officer (T7-8)
4	Mr. K. Jayarajan	Technical Officer (Stat.) (T6)
5	Mr. K.T. Muhammed	Technical Officer (T5) (Farm)
6	Mr. V. Sivaraman	Technical Officer (T5) (Farm)
7	Dr. C.K. Sushama Devi	Technical Officer (T5) (Lib.)
8	Ms. N. Prasannakumari	Technical Officer (T5) (Hindi Translator)
9	Mr. A. Sudhakaran	Technical Officer (T5) (Artist-cum Photographer)





### Administration

1	Mr. V. Mohanan	Admn. Officer – from May 2011
2	Mr. K.G. Jegadeesan	Asst. Fin. & Accts. Officer
3	Mr. C.Venugopalan	Asst. Admn. Officer
4	Mr. R.N. Subramanian	Asst. Admn. Officer
5	Ms. P.V. Sali	Private Secretary

### IISR Experimental Farm, Peruvanamuzhi

#### Scientific

1	Mr. P.A. Mathew	Principal Scientist (Horticulture)
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#### Technical Officers

1	Mr. V.K. Aboobacker Koya	Farm Supdt. (T9)
2	Mrs. E. Radha	Technical Officer (T6) – from 23 Sept. 2011
3	Mr. N.A. Madhavan	Technical Officer (T5)
4	Mr. K. Kumaran	Technical Officer (T5)

### Krishi Vigyan Kendra

#### Scientific

1	Dr. T. Arumuganathan	Programme Coordinator – from 29 April 2011
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#### Technical Officers

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

### IISR Cardamom Research Centre, Appangala

#### Scientific

1	Dr. S.J. Ankegowda	Sr. Scientist (Plant Physiology)
2	Dr. R. Senthil Kumar	Sr. Scientist (Horticulture)
3	Dr. C.N. Biju	Scientist (Plant Pathology)
4	Dr. T.R. Usha Rani	Scientist (Biotechnology) – up to 24 May 2011
5	Dr. R. Praveena	Scieintsit (Plant Pathology)

#### Administrative

1	Mr. P. Muraleedharan	Asst. Administrative Officer – from Aug. 2011
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## WEATHER DATA 2011

### IISR Experimental Farm, Peruvannamuzhi

Month	Temperature (°C)		Rainfall (mm)	Rainy days
	Maximum	Minimum		
January	33.70	18.61	17.0	1
February	34.96	18.35	12.0	1
March	35.32	22.09	16.2	1
April	34.18	22.46	206.2	15
May	34.38	24.09	203.4	10
June	27.66	23.09	1519.6	29
July	27.64	22.90	1058.8	29
August	28.06	23.06	873.8	28
September	29.38	22.53	642.2	19
October	31.00	22.19	267.2	13
November	32.75	21.71	88.2	7
December	33.96	20.38	3.0	1
<b>Average/Total</b>	<b>31.92</b>	<b>21.79</b>	<b>4907.6</b>	<b>154</b>

### Cardamom Research Centre, Appangala

Month	Temperature (°C)		Rainfall (mm)	Rainy days
	Maximum	Minimum		
January	26.4	14.0	-	-
February	29.1	11.4	13.8	1
March	30.3	13.7	24.8	2
April	31.6	15.4	117.2	8
May	29.5	17.6	61.6	4
June	25.1	18.2	644.8	25
July	23.9	21.9	616.2	30
August	23.91	16.1	625.4	30
September	24.7	16.5	424.0	20
October	27.0	17.7	142.3	13
November	26.7	16.2	71.4	6
December	27.2	14.0	-	-
<b>Average/Total</b>	<b>27.11</b>	<b>16.05</b>	<b>2741.5</b>	<b>139</b>

