RESEARCH HIGHLIGHTS
2011-12

Indian Institute of Spices Research
Kozhikode

Research Highlights
2011-2012

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

Correct citation
Indian Institute of Spices Research, Research Highlights 2011-12, Calicut

Publisher
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Indian Institute of Spices Research, PB No 1701, Marikunnu P.O.
Calicut 673 012, Kerala, India.
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May 2012

Printed at
KT Printers, Mukkam

PREFACE

The Research Highlights for the year 2011-12 presents the summary of achievements of the Indian Institute of Spices Research (IISR). The institute enriched and maintained its germplasm on all the mandate crops with additions from centers of All India Coordinated Research Project 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. Comparative gene expression analysis indicated expression of osmotin and beta 1,3 glucanase genes in *Piper colubrinum* and *P. nigrum*. An isolate of *Phytophthora* (Is. No. 98-93) infecting black pepper was completely sequenced using next generation sequencing platform.

Promising nematode resistant accessions were identified in ginger and turmeric for multi location trials. The target yield equation for soil test based fertilizer recommendation for a fixed yield target of black pepper, ginger and turmeric were developed and validated. Seedling progenies of turmeric with curcumin content above 4% were identified. Protocol for real-time PCR based detection of *Cardamom mosaic virus* (CdMV) and *Banana bract mosaic virus* (BBrMV) in cardamom has been standardized. A new species of EPN belonging to the genus *Oscheius* was identified. The combination product of carbendazim + mancozeb was found to be promising in black pepper and hexaconazole was promising in cardamom, in managing anthracnose and leaf blight diseases. A database on *Radopholus* genus called RADOBASE was developed and launched.

Kisan Mela and Technology Week were organized at IISR Chelavoor during second week of February 2012. The institute participated in three state level exhibitions, fairs and one district level exhibition. Media visits were organized and technologies were popularized 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 also appreciate the editors for having compiled and brought out this compilation.

Calicut
30 April 2012

M Anandaraj
Director

CONTENTS

BLACK PEPPER

CROP IMPROVEMENT

Genetic resources

The black pepper germplasm assembled at the conservatory are maintained in the nursery and field genebank. The present status is 2936 accession (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. Germplasm collection 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* (Fig.1) 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 (Fig. 2) was also collected from this area. In addition, two cultivar accessions from Assam were collected and added to the germplasm. One hundred and thirty accessions were characterized for eight morphological characters.

Fig. 1 *P. hapnium* (♂) collected from Sabari hills

Fig. 2 *P. nigrum* (lemonish flavor) collected from Sabari hills

Breeding black pepper for resistance to pollu beetle

The two hundred hybrid progenies (Subhakara × Coll. No. 816) are maintained in the field. Fifty hybrid progenies were screened for pollu infestation and all the progenies were susceptible.

Screening mapping population for Phytophthora resistance

Fifty seven lines selected as association mapping population were screened using leaf and stem inoculation methods. Two genotypes, Acc. No. 1324 (Aimpiriyan) and HP 780 (Perambramundi × 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 trancriptomics 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 full length genes discovered through transcriptome data analysis was found to have coding sequences corresponding to 913 and 303 amino acids. Both of the genes tested

were specifically up-regulated during challenge with *Phytophthora* and peak expression was observed at 2 hours and 24 hours post inoculation (hpi), respectively. 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* was found to be related to plant disease resistance genes.

Comparative gene expression analysis indicated very high expression of osmotin and β-1,3 glucanase genes in *P. colubrinum* compared to *P. nigrum*. Extensive transcriptional activity of other genes like peroxidase was also found. In the qPCR analysis done on *P. colubrinum* samples, the expression of β-1,3 glucanase gene was found to be at its peak at 48 hpi with *P. capsici* whereas the expression of osmotin was at its peak at 24 hpi.

Genetic fidelity testing

Ten TC samples (5 cultures and 5 hardened plants) of black pepper were received for genetic uniformity testing from TERI, New Delhi. The Samples tested with 5 ISSR primers short listed for black pepper and 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 seen with *P. hamiltoni* as scion giving 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*.

CROP PRODUCTION

Allelopathic effect of tree standards

In the green house study on the allelopathic effect of tree standards on growth of black pepper, the data taken on 180 days after planting (DAP) suggested that irrespective of the tree species plant height decreased at higher concentrations of leaf and stem extracts of 50 and 100%. The plant height was maximum at 12.5% and 25% concentration but decreased significantly at 50% and 100% concentrations. Results on number of leaves, root length and fresh weight also followed an identical trend.

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.

CROP PROTECTION

Morphological diversity of Phytophthora isolates

Phytophthora isolates (166) were characterized based on colony and sporangial morphology. The colony morphology showed eight different patterns *viz.*, chrysanthemum, modified chrysanthemum, floral, floral with cottony mycelia, stellate, stellate with cottony mycelia, cottony and cottony with concentric rings in carrot agar media (Fig. 3), while the sporangia showed nine different types of morphology *viz.*, aciculate, elliptical, elongated, ovoid, bovid, obpyriform, ovoid-obpyriform, globose and distorted shapes.

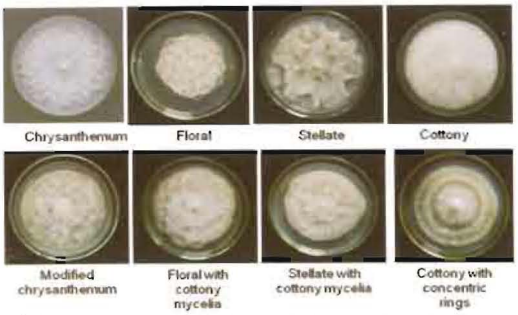


Fig. 3 Colony morphology of *Phytophthora* isolates

Molecular profiling of Phytophthora isolates from black pepper

Thirty six SSR primers were designed from the EST database of *P. capsici* and were screened for their polymorphisam among isolates. Twenty nine of the 36 primers amplified the expected amplicon. A total of 35 alleles were scored. The dendrogram generated showed that the similarity coefficient ranged from 79-100%. Cluster analysis using the un-weighted pair-group method with arithmetic averages (UP-GMA) divided the isolates into two major clusters.

Molecular diversity of 82 *Phytophthora* isolates from black pepper 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. citophthora*, 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* have been carried out. Functional annotation of SNPs in *P. capsici* was carried out and deleterious nsSNPs were identified through *in silico* analysis.

Management

Laboratory and greenhouse trials

New chemicals such as Fenamidone-Mz (Sectin), Famoxadone-Cymoxanil (Equation Pro), Cymoxanil-Mz (CurzateM8) and Dimethomorph 50% (Acrobat 50) were evaluated *in planta* against *P. capsici* in comparison with Metalaxyl- Mancozeb (Mz) (1250 ppm) and among them Metalaxyl-Mz at the recommended dose was the best in preventing foliar infection and root infection on par with Fenamidone - Mz (450 ppm).

Twelve phytochemicals from phenyl propanoid pathway of black pepper were docked to modeled β-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 black pepper rooted cuttings in a greenhouse study.

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 trials

Field evaluation of trial 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 the IISR 6.

Viral disease

Development of Real-time PCR for detection

SYBR green based real-time PCR was developed for detection of *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV) in black pepper. The protocol involves 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.

Seed transmission

Studies to know the location of the virus in black pepper 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 parameters

Observations on anthracnose incidence and weather variables *viz.*, maximum temperature, 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 both rainfall and number of rainy days had positive correlation with the disease progression.

Survival of pathogen

Survival of *Colletotrichum gloeosporioides* infecting black pepper in the infected plant part (leaves) was studied under laboratory, greenhouse and field conditions. The fungus survived in the infected plant debris of black pepper for about 3 months under field conditions and under laboratory conditions for more than 4 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 (Fig. 4). 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.

Fig. 4 Formation of microsclerotia under *in vitro* conditions on a glass slide

Management

The efficacy of different fungicides was evaluated against anthracnose disease 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

The total essential oils, phenols and surface wax were estimated in berries of three resistant black pepper accessions (816, 841 and 1114) and one variety susceptible (Panniyur 1) to pollu beetle. 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 µg/g and varied from 0.735 to 1.011 µg/g in resistant accessions. The surface wax in the resistant accessions of immature berries varied from 0.4782 to 0.6917 µg/g and in susceptible accession it was 0.2926 µg/g.

CARDAMOM

CROP IMPROVEMENT

Germplasm characterization

Cardamom field gene bank enriched with 17 new accessions (from RRS, Mudigere) bringing the total to 562. Morphological characterization has been recorded in 50 accessions. 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.

Molecular characterization

Molecular profiles were developed for 100 accessions of small cardamom germplasm using 25 ISSR markers for studying the genetic diversity and dendrogram of similarity was prepared using NTSYS. The study clearly indicated the diversity among small and large cardamom accessions selected for developing core collections. Around 270 clones from cardamom genomic DNA were isolated and sequenced using hybridization – enrichment method for generating microsatellites. Twenty four primers were designed and 20 Primers gave good amplification in small cardamom. The sequence information obtained from 200 clones from small cardamom and 100 clones from large cardamom are being processed for developing primers for more SSRs.

Evaluation of cardamom germplasm 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 IC 547184. GG contained 20.8% 1,8- cineol and 48.1% α-terpinyl acetate whereas IC 547184 contained 24.1% 1,8- cineol and 49.8% α-terpinyl acetate. These two accessions showed similar composition except that GG contained relatively higher levels of limonene and geraniol compared to IC 547184.

Evaluation of hybrids

Analysis of 4 successive crop yields of 29 F_1 hybrid progenies of Preliminary Evaluation Trial-I and II resulted in identifying following hybrids: 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) to be promoted to coordinated varietal trials.

Genetic fidelity testing

Ten TC samples (5 cultures and 5 hardened plants) of large cardamom were received for genetic uniformity testing from TERI, New Delhi. The samples tested with 5 ISSR primers short listed for small cardamom were found to have partial instability in three lines with one of the five primers tested.

CROP PRODUCTION

Performance of elite lines under moisture stress

Twelve short listed genotypes along with three checks were evaluated for growth and yield parameters under stress. Significant variation was recorded between genotypes for growth and yield parameters. IC 584058 (APG 474) recorded early yield, good setting and bold capsules. It recorded 32 total tillers per clump, 44 panicle per clump, 176.8 capsules per panicle and 3889.6 capsules per clump with more than 80% bold capsules (>8 mm).

Fig. 5 IC 584058 (APG 474)

IC 584078 (GG × 893) a multibranched panicles genotype recorded 85 panicles per clump, 71.2 capsules per panicle, 6072 capsules per clump with more than 50% bold green coloured capsules (>8 mm), and took longer time for leaf folding when exposed to sunlight.

Fig. 6 IC 584078 (GG × 893) a multibranch type

CROP PROTECTION

Viral diseases

Development of Real-time PCR for detection

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

Seed transmission studies were carried out using 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

Incidence in the field

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 a high incidence and severity of rhizome rot disease 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.

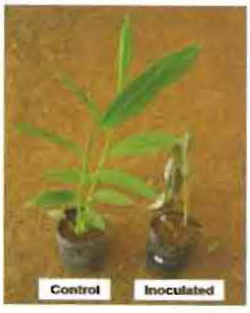


Fig.7 Pathogenicity of *Fusarium* sp. on IISR Appanagala 1

Pathogenicity and characterization

Pathogenicity of 10 *Fusarium* isolates was tested on Appanagala 1 (Fig.7) and Appangala isolate caused complete wilting of the seedling within 6 days after inoculation. Morphological characterization of 10 *Fusarium* and 8 *Rhizoctonia* isolates was completed.

Leaf 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 3 months under field conditions. Under laboratory conditions the fungus survived for more than 4 months.

Thrips

Host plant resistance

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.

Bacterial endosymbionts

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*.

Management

Eleven insecticides and natural products were evaluated against cardamom 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 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

CROP IMPROVEMENT

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, (Fig. 8) 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%.

Fig. 8 *Curcuma longa* collected from Thekkadi forests

Promising lines

The shortlisted seven nematode tolerant accessions of turmeric were evaluated for morphological and yield characters and Acc. 79 and Acc. 48 were found to be promising. About 215 seedling progenies were analyzed for curcumin, oil and oleoresin contents. Thirteen seedling progenies showed curcumin content above 4%. Seedling progeny 389/1 showed above 5% curcumin consistently for 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 reported for turmeric. 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 turmeric samples were received from one of the planters to test whether they are pure seed material of variety Prathibha. The samples were profiled with 4 ISSR primers. The results showed that of the 20 lines tested only 5 were similar to Prathibha.

CROP PRODUCTION

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

Organic farming

The rhizome yield was higher under integrated systems followed by organic and chemical systems. Significantly higher oleoresin and curcumin contents were recorded under organic on par with integrated management. Under organic management Alleppey Supreme recoded 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 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 (Fig 9). Application of one or two foliar spays 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/ $3m^2$ respectively and also increased the curcumin content significantly (4.86 – 6.08%).

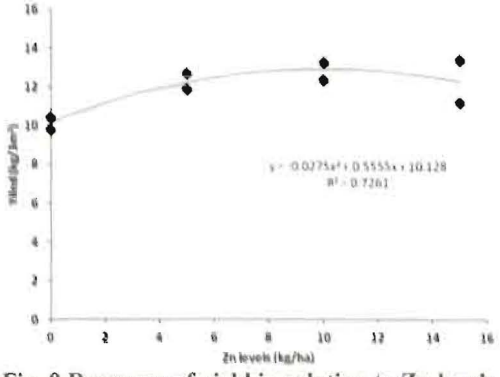


Fig. 9 Response of yield in relation to Zn levels

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, *Salvia miltiorrhiza*.

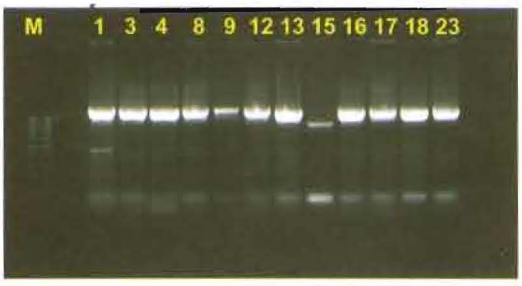


Fig. 10 Clones of *pal* Primer TA1.33F/ TA1.33R in PTZ57R/T (M: Mass ruler; 1-15: PTZ57R/T positive clones)

Essential oil profile of related species and released varieties

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 β-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%), β-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%), α-terpineol (2.56%), curzerene (5.2%), and β-elemene (4.64%) in *C. caesia*. Essential oil content in 13 varieties of turmeric, namely, 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 α-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%), β-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

CROP IMPROVEMENT

Genetic resources

Wild germplasm exploration and collection were carried out in the Thekkady forests, Idukki district, Kerala. A putative wild type ginger (*Zingiber officinale)* was collected and conserved in the *ex-situ* gene bank at IISR, Peruvannamuzhi. The collection is characterized by very small rhizomes, reduced tillers, medium stature and less abundance (Fig.11).

Fig. 11 *Z. officinale* collected from Thekkadi forests

Promising lines

The shortlisted five nematode tolerant accessions of ginger were evaluated for morphological and yield characters and Acc. 219 was found to be promising with high yield.

Pooled analysis of the yield and quality data of exotic ginger accessions indicated the superiority of Accs. 578, 581 and 593.

About 116 M_6V_6 and 181 M_5V_5 generation plants of irradiated varieties were maintained in pots. Samples of these materials were screened against *Ralstonia solanacearum.* After three rounds of inoculation two mutants *viz.*, R 0.9-14 and M 0.5-1(1) were found to survive the inoculation.

CROP PRODUCTION

Evaluation of PGPR strains for nutrient mobilization

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 indicating more favorable conditions for microbial growth.

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. 3.75/ bed for N, Rs. 1.30/ bed for P and Rs. 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

Partitioning studies in three different varieties *viz.*, IISR Varada, IISR Rejatha and IISR Mahima showed that at 50 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 μ moles $m^{-2} s^{-1}$) 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).

CROP PROTECTION

Bacterial wilt

Characterization of pathogen

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 (*pps*A, *adk*, *gap*A, *gdh*A, *gyr*B) and virulence genes (*hrp*B, *fli*C 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. 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 phylogentic groups.

Management

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 β subunit) gene sequencing. Sequence information showed that all the potential isolates belonged to *Streptomyces* species.One isolate was identified as *Kitasatospora setae*.

Rhizome rot

Management

Field experiments indicated that *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB 68) were effective for disease control and plant growth promotion.

Shoot borer

Biochemical characterization

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^2 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^2, respectively.

Mass production of entomopathogenic nematodes

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 (Fig. 12).

Evaluation of infectivity of EPNs

Two isolates of EPNs *viz.*, *Steinernema abbasi* (NBAII SA 01) and *Heterorhabditis indica* (NBAII Hi 1) obtained from NBAII,

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 change.

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 to about 15%. Curcumin, oleoresin, total phenol and antioxidant property did not show any reduction.

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 in both methods.

Nutraceutical properties of bioactive compounds

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 (Fig. 14).

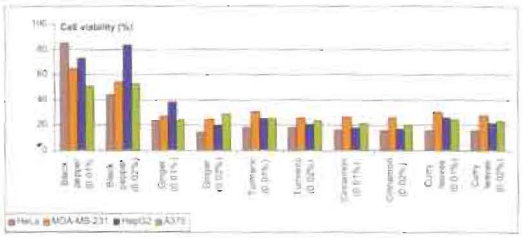


Fig.14 Cell viability of four cancer cells treated with essential oil of spices

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

Turmeric leaf oil and *Cinnamomum 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 (Fig. 15).

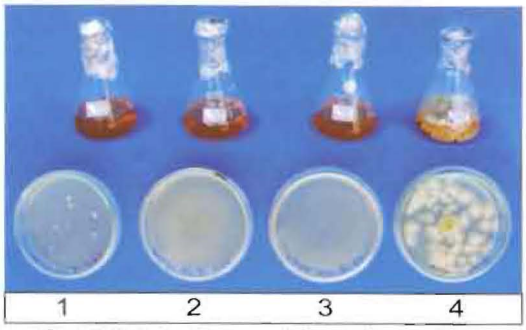


Fig.15 Effect of turmeric leaf oil on growth of *Aspergillus flavus* (1- 1.0 %, 2- 1.5%, 3- 2.0%, 4- control)

BIOINFORMATICS

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 (Fig. 16).

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.

Fig.16 *Phytophthora* Genome Database

EXTENSION AND TRAINING

During the year, 897 farmers (365 from within district, 220 from the state and 312 from outside state) and 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 (37 trainees) and for a group of field extension officers of Department of Horticulture, Kodagu district, Karnataka.

During the year planting materials worth ₹ 182290 and publications worh of ₹ 18770 were distributed. 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

The project envisages analyses of 17,069 soil samples of 87 Panchayats of Calicut district, analyses for pH, OC, major-, secondary- and

micro-nutrients, uploading the data into www.keralasoilfertility.net. Subsequently nutrient advisory cards will be generated followed by identification of production potential of each AEZ, development of nutrient management plans for our AEZ, and development of GIS maps for integration with the NREDB database generated as part of ISRO funded project. As on date, 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.

Farming models to address the agrarian crisis of Wayanad District

About 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 26th May 2011. 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 two 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. Fifteen percent 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 kg/ha among which >35% of samples 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 was organized from February 16 to18, 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 Selvan, 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, Calicut 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 17 February 2012. Ms. R. Ushamani, Principal Agricultural officer, Calicut, inaugurated the programme and Dr. S. Ramachandran, Director, Regional Science Centre and Planetarium, Calicut, presided over the function (Fig. 17). 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. 17 PPV & FR programme: inaugural 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 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.

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 (Fig. 18)

Fig. 18 New PGPR mix for spices

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, black pepper and 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 60th and 90th 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

Fig. 19 New nutrient mix for spices

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. 19).

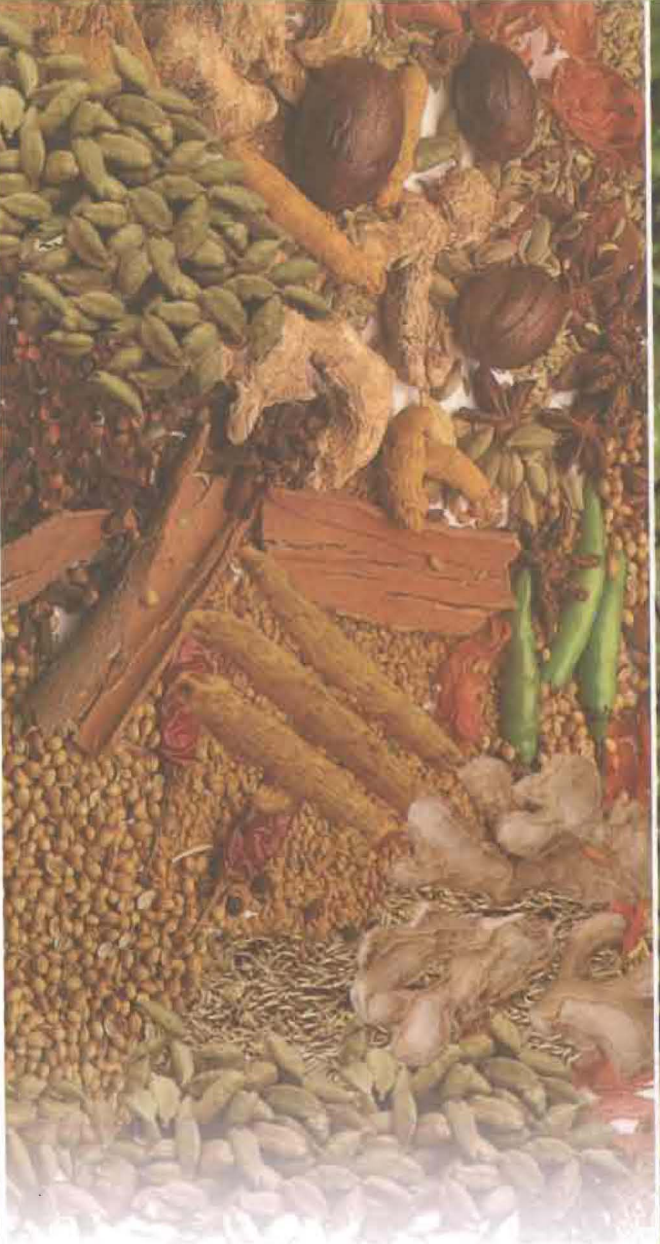
HUMAN RESOURCE DEVELOPMENT

- One month summer training on Biochemistry, Biotechnology and Bioinformatics was conducted for 14 M.Sc. students during 3rd May – 4th 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 from September 12-25, 2011(Fig. 20). The national training, exposed the use of genomic technologies along with genetic and bioinformatics approaches for identifying allelic variations and to dissect trait-gene associations.

Fig. 20 Dr. HP Singh, DDG (Horticulture) Chairing the valedictory function of the training



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