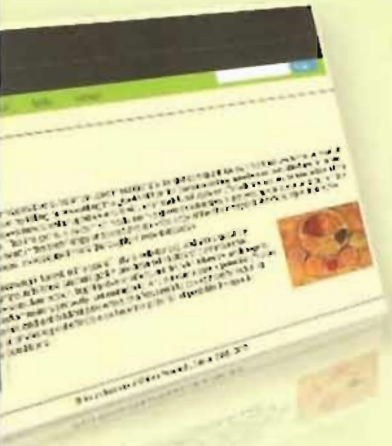
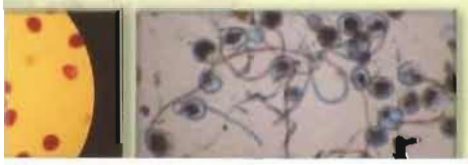


Research Highlights 2008-09

Indian Institute of Spices Research
Calicut



Research Highlights

2008–09

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PREFACE

The Research Highlights of IISR for the year 2008-09 assumes great significance in the light of the fact that the EFC of the Institute has been cleared. Noteworthy feature of the EFC has been the recognition of the contribution of IISR in the high quality research pertaining to wilt diseases affecting crops. An outlay of 19.65 crores has been ear-marked with IISR as the lead centre with 20 centres for *Phytophthora, Fusarium and Ralstonia* research.

During the year a National Seminar on *Piperaceae* was conducted besides two training programmes (a Winter School and a Short Course) funded by ICAR. The Institute enriched its germplasm on all the mandate crops and with two more accessions having been registered with NBPGR. The PPV & FR Authority has finalized the DUS guidelines and is in the process of getting it cleared through a Gazette Notification. The impact of various technologies developed is being studied by the Institute. The level of adoption for control of fungal diseases was around 57%. Intensive studies are being carried out to pin-point the refinement needed for further improvement of technologies.

I consider it as my duty to place on record the encouragement and support given by Dr. Mangala Rai, Director General, ICAR for having considered IISR as a Nodal Agency for the important outreach programmes. This would not have been possible but for the strong encouragement and guidance we received from Dr. H.P. Singh, Deputy Director General (Horticulture). He was a source of strength for us. We are also grateful to Dr. Umesh Srivastava, ADG (Hort. II) for all the support he gave to us. I appreciate the efforts taken by the staff of this Institute irrespective of the cadre, be it administrative, supporting, technical or scientific, in taking our research to its logical conclusion. I appreciate the editors for having compiled and brought out the Highlights in time.

Calicut
02-04-2009

V.A. Parthasarathy
Director

CONTENTS

BLACK PEPPER

CROP IMPROVEMENT

Germplasm maintenance

Two thousand five hundred and seventy five accessions (1266 wild accessions, 1300 local cultivars and nine exotic accessions) are maintained in the black pepper germplasm conservatory. An alternate germplasm conservatory for wild *Piper* accessions was established at CRC, Appangala with 238 accessions in the field gene bank. Survey was conducted for collecting germplasm from Charmadi, Mudigere, Khalsa and Kudremukh forests and collected 32 *Piper* accessions. Bush peppers of important local cultivars and wild relatives were developed and planted at IISR, Calicut. Herbarium specimens of black pepper accessions were prepared and preserved in the herbarium facility.

Registration of germplasm accessions

Two accessions were registered with ICAR for their unique characters. They are:

1. INGR 8099- *Piper thomsonii* (IC- 398863) - for its unique character for sex change (from male to bisexual plant).
2. INGR 8100- *Piper nigrum* (IC- 563950) - A novel spike variant with proliferating spikes.

CROP PRODUCTION

Irrigation

An experiment conducted in a planter's field at Madikeri revealed that irrigating pepper vines once in a fortnight from March to May months @ 50 litres/vine can enhance pepper yields substantially. The mean dry yield obtained was 6.8 kg/vine in irrigation treatment as against 3.25/kg vine under rainfed condition.

Intercropping

Among the various crops tried for a period of three years, crops such as ginger (Varada), tapioca (Sreejaya), coleus (Nidhi), amorphophallus (Gajendran) and hybrid napier (Co-3) were found suitable for intercropping in black pepper garden which is more than 15 years old.

Nutrient requirement for targeted yield

In black pepper 5, 7.5 and 10 kg dry yield/vine was targeted and fertilizer doses for the same based on soil fertility was calculated and imposed in two splits in Mrigarajendra estate, Madapur, Madikeri. The yield targets could be achieved with minimum deviation from the targets. The recorded yield levels were 5.5, 7.3 and 8.2 kg/vine in the targets of 5, 7.5 and 10 kg/vine, with a deviation of +11%, -2.2% and -18.0% respectively. The yield increase as compared to the control was 39-104%.

Figure 1. Black pepper fruit setting under summer (March to May) irrigation

Nutrients addition by cover crops

Nutrient addition by cover crops *viz.*, cowpea and horse gram for three seasons from September to May was estimated in the black pepper plantation. The crop sequence with cowpea alone could add 95.04, 5.81, 27.46, 24.29, 3.96 kg N, P, K, Ca, Mg, per ha., respectively, whereas, a sequence with horse gram alone could contribute 26.08, 2.36, 15.90, 7.58, 2.48 kg of N, P, K, Ca, Mg, per ha., respectively. A sequence with cowpea followed by horse gram or vice-versa could contribute less than the sequence with cowpea alone.

QUALITY

Constituents of essential oil of cultivars grown at two different locations

Germacrene-D and elemol were found to be the major constituents of leaf oil of black pepper cultivars from IISR Experimental Farm, Peruvannamuzhi, Calicut and from Pepper Research Station (PRS), Panniyur. β-caryophyllene was high in berries at both locations and it showed more variability in berries compared to leaf samples. The concentration of β-caryophyllene in leaf oil varied from 2.03 to 8.47% and that of germacrene D from 4.08 to 44.2%. Highest germacrene D content was found in Panniyur-3 followed by Panniyur-2, Panniyur-6, and Panniyur-7. Another major constituent in the leaf oil of many cultivars was elemol and its concentration varied from 2.37 to 55.2%. Highest concentration of elemol was found in Vatamunda followed by Neelamundi and Angamali. A few cultivars had α-bisabolol, eudesmol, nerolidol and farnesol.

Controlled atmosphere storage and quality

Black pepper samples stored with a moisture content of 10% under controlled atmosphere (90% nitrogen + 10% oxygen) for 480 days did not show significant variation in essential oil, oleoresin and piperine contents compared to control.

CROP PROTECTION

Phytophthora resistance

Open pollinated progeny of IISR Shakthi (04-P24 -1) continued to show resistance to root infection by *P. capsici* with repeated inoculations. Out of 155 hybrids, 68 cultivars and 13000 seedling progenies screened, three op progenies *viz.*, HP 1533(2), HP 1533(3) and 04-P24-1; four hybrids, HP 449, HP 490, HP 521, HP 1375 and one cultivar C 1530 were found moderately resistant. The promising lines showing moderately resistant reaction towards *P. capsici* are under field evaluation. Among the eight wild accessions screened against *P. capsici*, *R. similis* and *M. incognita*, accession 3362 (*P. ornatum*) showed resistance towards *Phytophthora* and also towards both the nematodes.

Phytophthora management

Biological control: Endophytic and rhizospheric biocontrol agents *viz.*, Bp 35, Bp 25, Bp 17, TC 10, IISR 853 and IISR 6 are being evaluated in an integrated management trial under field conditions against *P. capsici*, *R. similis* and *M. incognita*. The observations on six month old plants indicated that the treatments TC 10 + metalaxyl-mz and IISR 853 + metalaxyl-mz are promising.

Evaluation of new chemicals against *Phytophthora*: New chemicals such as benzoic acid, salicylic acid (a.i. from *Chromalaena odorata*), potassium bicarbonate, captan-hexaconazole and carbendazim-mancozeb were tested *in vitro* against *P. capsici*. Captan-hexaconazole was found to be completely inhibitory at 50 ppm. Benzoic acid and salicylic acid were inhibitory at 100 and 200 ppm respectively. Minimum inhibitory concentration for potassium bicarbonate and carbendazim-

mancozeb were 1000 ppm and 500 ppm, respectively.

Management of anthracnose disease

In the disease management trial with combination of fungicides (1% Bordeaux mixture, hexaconazole, carbendazim, potassium phosphonate, mancozeb) and biocontrol agents (*Trichoderma harzianum* and *Pseudomonas fluorescens*) conducted in hot spots of anthracnose and spike shedding under coffee + black pepper cropping system, the treatment with basal application of *T. harzianum* and aerial spray with 1% Bordeaux mixture was superior. No foot rot incidence was noticed in this treatment compared to 22.6 % mortality in untreated control. This treatment effectively reduced foot rot incidence also. Under field conditions, the anthracnose pathogen survived beyond 8 months in the infected debris.

Management of diseases in black pepper nursery

An experiment was conducted with solarized potting mixture, having 12 different treatments including control *viz.*, COC 0.2% + phorate, carbendazim 0.2% + phorate, metalaxyl-mz 0.125% + phorate, potassium phosphonate 0.3% + phorate, carbendazim-mz 0.25% + phorate, mancozeb 0.25% + phorate, *T. harzianum* + phorate, IISR 853, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, IISR 6 and control (ordinary potting mixture alone) to study their efficacy in controlling nursery diseases. The observations on the uprooted poly bag plants recorded after six months indicated that more than treatments or bag size, moisture content is playing a major role in root infection. No *Phytophthora* infection could be noticed in any of the treatments or control where the moisture content of soil in the poly bag was less than 10%. Absolutely no infection was noticed in control.

VIRUSES

Distribution of the viruses during different seasons

To know the variation in virus titre, virus infected black pepper plants belonging to five varieties were tested by DAS-ELISA for PYMoV and CMV at monthly intervals. Results indicated that the concentration of both the viruses varied during different months of the year. The concentration of both the viruses was found to be higher during October to January.

Variability in *Piper yellow mottle virus* (PYMoV) isolates

A portion of ORF I and ORF III from four isolates representing different geographical regions (Calicut, Idukki and Wayanad districts of Kerala and Kodagu District of Karnataka) were cloned and sequenced. Analysis using ORF I sequences showed high variability while OFR-III sequences were highly conserved among isolates.

Identification of the *Badnavirus* infecting *Piper longum* and *P. betle*

A portion of ORF I and III of *Badnavirus* infecting *Piper longum* and *P. betle* were amplified, cloned and sequenced. Black pepper isolates of PYMoV shared an identity of 94–97 % with *P. betle* and 88–89 % with *P. longum* at the nucleotide level. Per cent identity of less than 60 was seen with other badna viruses used for comparison. Thus based on sequence identities, it was concluded that *Badnavirus* infecting *P. betle* and *P. longum* in India are strains of PYMoV.

INSECT PESTS

Field screening for pollu beetle (*Longitarsus nigripennis*) resistance

Black pepper germplasm accessions (113 cultivars and 72 hybrids) were screened for resistance to pollu beetle. Among the cultivars,

Acc. No. 35 recorded the highest berry damage (36.5%) followed by Acc. No. 88 (34.5%). The lowest damage was on Acc. No. 1636 (1.75%) followed by Acc. No. 1423 (2.74%). Among the hybrids the damage was highest on Acc. No. 861 (44.4%), followed by Acc. No. 1471 (41.5%). The lowest damage was recorded on Acc. No. 1388 (4.49%), followed by Acc. No. 1726 (5.74%).

Incidence of *Erythrina* gall wasp (*Quadrastichus erythrinae*)

Surveys were conducted in 276 black pepper (*Piper nigrum*) gardens in 13 taluks in 4 districts (Idukki, Kozhikode, Kannur and Wayanad) in Kerala and 3 taluks in 1 district (Kodagu) in Karnataka, during April to July 2008, to study the incidence of *Erythrina* gall wasp (*Quadrastichus erythrinae*), on *Erythrina* spp., used as standards for trailing black pepper vines. *Erythrina* gall wasp incidence was observed in all the locations surveyed and the percentage of trees infested by the pest was significantly higher in Wayanad District (59.6%) that was on par with Idukki (53.4%), Kodagu (51.8%) and Kannur (39.1%) districts. The percentage of twigs infested by the pest was also significantly higher in Wayanad District (39.7%) that was on par with Idukki (35.5%), Kodagu (33.4%) and Kozhikode (31.6%) districts. The severity of incidence varied on various species / types of *Erythrina* and was significantly higher in *E. variegata* (white-thorn type) wherein a mean of 91.8% trees and 66.8% twigs were infested by the pest. The surveys indicated that infestation of *Erythrina* spp. by the gall wasp has become one of the major constraints in black pepper production in Kerala and Karnataka, in recent years.

NEMATODES

Phenyl propanoids in nematode resistance

Activities of phenylalanine ammonia lyase (PAL) and caffeic acid-O-methyltransferase (COMT) increased in roots and leaves of susceptible black pepper variety (IISR Sreekara) on infection with *Radopholus similis* indicating the role of phenyl propanoids in black pepper – nematode interactions.

Sequence similarity

Sequencing of the polymorphic 650 bp fragment cloned from nematode tolerant accession HP 39 of black pepper showed maximum similarity to acetyl-CoA carboxylase and not to any genes attributed for nematode resistance.

CARDAMOM

Germplasm characterization

Fifty accessions were characterized during the year thus making total number of characterized so far to 350. A total of 442 accessions have been maintained in the *ex situ* gene bank at Cardamom Research Centre, Appangala (Malabar 278, Mysore 73, Vazhukka 63 and others 28).

Evaluation of hybrids

Evaluation of hybrids under PET 1 and PET 2 during 2008-09, led to the identification of the following hybrids *viz.*, CCS-1 x RR-1, RR-1 x CCS-1, MB-5 x GG, ASH, NKE 19 x GG, GG x NKE 19. In MLT trials, SAM, CP-4 and NHY-10 performed better compared to others.

Drought tolerance

Evaluation of four cross combinations of cardamom (hybrids and selfed seedlings) for drought tolerance in field revealed that Green Gold and its cross combinations were better in terms of growth and yield characters under stress compared to other crosses. 893 x RR1, GG x RR1, CCS1 x GG, GG x 893 and CCS1 x GG took more time to fold leaves (leaf rolling) under open light than other crosses.

Quality evaluation

Sixty two accessions of cardamom, including 5 hybrids were analyzed for seed content, essential oil yield and essential oil composition. The cardamom capsules contained 55- 82% seeds and 4.0-6.5% essential oil. The accession Nos. VA-1, MA-18, IC 391657, IC 547147, IC 547144, IC 349652, IC 349633, IC 547153 and IC 547220 recorded 66-74% seed content and 6.5% essential oil yield. The major constituents of the oil, namely, 1, 8-cineole varied from 22 to 40% and α-terpenyl acetate from 32 to 47%. The accession numbers, GG x NKE 12, NKE 19, OP27, MA 29, IC 547147, IC 547154 and IC 547144 were identified as 1, 8-cineole-rich accessions (>35%) and PC, CP-4, NKE 12 x GG, NKE 19 x GG, GG x NKE 19 and GG suckers were rich in α-terpenyl acetate (>45%).

Soil quality

Soil samples collected from various cardamom based cropping systems *viz.*, cardamom + nutmeg, cardamom + allspice, cardamom + cinnamon, cardamom alone, cardamom + coffee and cardamom + arecanut were analyzed for various soil quality parameters. The results revealed that the component crops had little influence on the various physico-chemical and biochemical parameters studied. Soil pH varied between 4.8-5.2 and organic C between 1.9-2.7%. Available N levels ranged between 121-148 mg/kg, Bray P between 21-41 mg/kg, and available K between 212-338 mg/kg. Among the micronutrients, DTPA Zn was low at all the sites (0.72-1.04 mg/kg).

Katte virus purification and detection

Eighteen *Katte* isolates were collected from Karnataka and Kerala states and established under insect proof glass house conditions. Protocol for purifying the causal virus (*Macluravirus*) of *Katte*/mosaic disease of cardamom has been standardized. Protocols for RNA isolation (RT-PCR) for detecting *Macluravirus* from the leaves obtained from *Katte* affected cardamom plants has been standardized. The virus can be detected at 7µl template concentration from the extracted samples.

Screening for resistance to diseases

Fifty eight cardamom accessions were screened for leaf blight, rhizome rot and leaf blotch resistance under natural field conditions. Based on their reaction towards leaf blight and rhizome rot diseases, the accessions were categorized into different groups by combining the information obtained from two consecutive years *i.e.*, 2007 and 2008. None of the accessions were found highly resistant to leaf blight disease. However, IC-349646 was found to be resistant to this disease with an average leaf blight incidence of 20%. Twenty three accessions exhibited highly resistant reaction to rhizome rot disease.

Figure 2. Detection of *Cardamom mosaic virus* (CdMV) by reverse transcription (RT) PCR in cardamom samples.
Lane 1: DNA size marker (500 bp ladder); Lanes 2-11: different cardamom samples from Karnataka and Kerala; Lane 12: healthy cardamom

TURMERIC

CROP IMPROVEMENT

Germplasm collection

Thirty four turmeric accessions are added to the germplasm repository during 2008-09, thus raising the total germplasm collections to 1026.

Development of microsatellite markers and characterization of *Curcuma* species

A total of 140 microsatellites containing genomic DNA fragments were isolated from turmeric (*Curcuma longa* L.) adopting the selective hybridization method with di and trinucleotide biotinylated probes. Expressed sequence tags (EST's) of turmeric hosted in the NCBI database were screened for type and frequency of class I (hypervariable) simple sequence repeats (SSR's). Eight polymorphic primers were identified for amplifying SSR containing ESTs. These primers were also transferable to 13 different species of *Curcuma*. The AE HPLC elution profiles of water soluble rhizome proteins of thirteen *Curcuma* species on UPGMA formed six major clusters with *C. malabarica* and *C. zedoaria* showing maximum similarity. The biologically active peptide turmerin isolated from all the 13 species of *Curcuma* showed highest concentration in *Curcuma sylvatica* (320 mg/100 gm).

Phenology and dry matter distribution

Turmeric varieties *viz.*, Prathibha, Alleppey Supreme, Suguna, Prabha and Kedaram on an average took 23.5, 18.5, 16.5, and 12.3 days for emergence and 98.4, 59.9, 56.7, and 48.5 days for producing first tiller under 30 April, 15 May, 30 May and 15 June planted crops, respectively. The dry matter distribution among root, rhizome, pseudo-stem and leaf was estimated by destructive sampling at fifth month after planting. On an average turmeric varieties recorded 8.10%, 60.7%, 8.90% and 22.29% dry matter in root, rhizome, pseudo-stem and leaf, respectively.

QUALITY

Controlled atmosphere storage and quality

Turmeric samples stored with a moisture content of 10% under controlled atmosphere (90% nitrogen + 10% oxygen) for 480 days showed minimal variation in essential oil and no variation in oleoresin and curcumin contents compared to control. Samples stored under controlled atmosphere were totally free from insect attack.

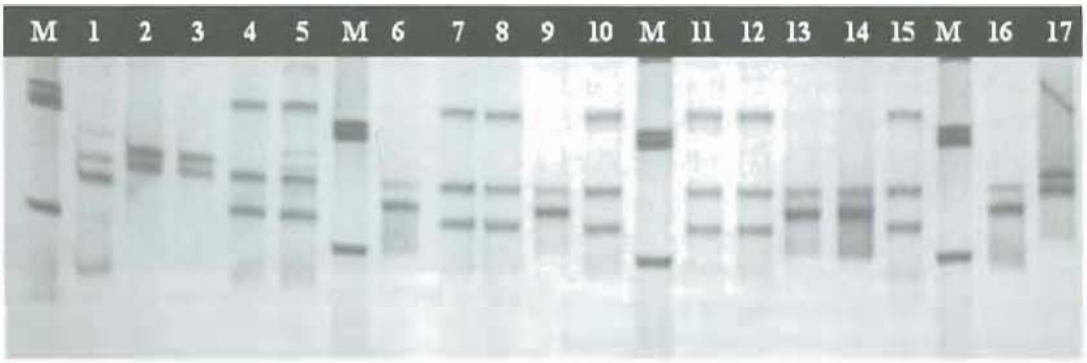


Figure 3. Polymorphic EST-SSR markers in turmeric accessions with primer CLEST SSR-02.
M: 50 bp DNA ladder; Lanes 1 to 17: turmeric accessions/varieties from various geographical locations (Agarthala, Alleppey, Alleppey Supreme, Amalapuram, Arunachal, Avanigadda, Ayur, Coimbatore, Dhagi, Diblugarh, Gaspani, Gorakhpur, Jobedi, Jorhat, Kasturi, Katergia, and Kedaram).

Influence of drying methods on quality

Turmeric varieties Prathibha and Alleppey were processed with and without boiling. Later, these samples were dried in hot air oven, reverse flow and sun dried and were evaluated for oil, oleoresin and curcumin. Processing with or without boiling or different drying methods did not lead to variation in oil, oleoresin and curcumin contents.

DISEASES

Rhizome rot

Management using *Trichoderma* : Twenty two isolates of *Trichoderma* spp. isolated from the turmeric rhizosphere were evaluated for their antagonistic potential against *P. aphanidermatum* causing rhizome rot of turmeric. Out of twenty two isolates, six isolates showed mycelial inhibition of above 70% *in vitro*. The highest inhibition by volatile metabolites was shown by CLT 118 (84.82%) and CLT 121(82.22%). The non volatile metabolites produced by CLT 102 (37.78%), CLT 107 (38.52), CLT 110 (46.30%) and CLT 114 (42.22%) were comparatively effective against the pathogen. CLT 114 from Bidar (Karnataka) and CLT 102 from Wayanad (Kerala) induced cytoplasmic coagulation of the pathogen in dual culture.

Management using chemicals: Field trial was conducted during 2007-09 in turmeric fields at Settiputhur, near Annur in Coimbatore Dist., Tamil Nadu, to study the efficacy of various chemicals to manage the disease as it was reported that the disease is a serious threat to turmeric farmers in the region. The trial consisted of 10 treatments *viz.*, copper oxychloride (0.25%), cheshunt compound (0.3%), Bordeaux mixture (1%), metalaxyl-mancozeb (0.125%), mancozeb (0.3%), potassium phosphonate (0.3%), carbendazim-mancozeb (0.3%), captan- hexoconazole (0.3%), carbendazim (0.5%) and control. Among the treatments, maximum disease reduction was observed with metalaxyl-mancozeb (44.5%) followed by copper oxychloride (36.5 %) when compared to control.

Molecular characterization of *Pythium* isolates from rhizome rot of turmeric

The combination of universal primers ITS1 and ITS4 were used to characterize pathogenic *Pythium* isolates (collected from turmeric growing tracts of southern states of Kerala) which generated a 900 bp fragment in PCR. The amplified products were digested with restriction enzymes *Msp* I, *Taq* I and *Alu* I for PCR-RFLP analysis. RFLP finger print data grouped the isolates into two main groups with 10% similarity.

Figure 4. *Trichoderma* CLT 107 antagonistic to *P. aphanidermatum*

SHOOT BORER (*Conogethes punctiferalis*)

Germplasm screening

Turmeric accessions (915 Nos.) were field screened against the shoot borer. The maximum shoot damage (80%) was observed on accessions 345, 525 and 882. Twelve accessions (Accs. 405, 444, 455, 692, 718, 880, 888, 897, 917,

979 and 995) recorded 75% shoot damage on them. Forty four accessions remained free from the shoot borer attack.

Natural enemies

The natural enemies of shoot borer (*Conogethes punctiferalis*) infesting turmeric were documented. The natural enemies documented during the crop season included *Apanteles taragamme* ?, an un-identified hymenopterous parasitoid and earwigs.

Management

Four insecticides (malathion 0.1%, carbosulfan 0.075%, imidacloprid 0.0125%, and lamda cyhalothrin 0.0125%) that were promising in the greenhouse were evaluated in the field at Peruvannamuzhi for their efficacy in the management of the shoot borer. The insecticides were sprayed at 21-day interval during July to November and the incidence of infested shoots was recorded at crop maturity during December. The trials indicated that all the insecticides except imidacloprid (0.0125%) were promising in reducing the percentage of shoots infested by the shoot borer.

GINGER

Maintenance of germplasm

Six hundred eighty accessions of ginger are being maintained in field germplasm conservatory. Twenty accessions were added to the germplasm repository during 2008-09.

Germplasm evaluation

Twelve selected exotic (Nepal) accessions along with check IISR Varada were evaluated for morphological and yield characters. Tillers/clump and rhizome yield varied significantly among the accessions. Maximum yield per clump was recorded in Acc. 578 with 9.82 kg/3m^2 which was on par with check (9.63 kg/3m^2). Evaluation of 13 high oil ginger accessions for morphological and yield characters revealed highest yield in Acc. 162, followed by Acc. 217 and Acc. 209. Yield varied from 5.26 to 8.74 kg/3m^2 bed in these accessions.

High pollen fertility accession

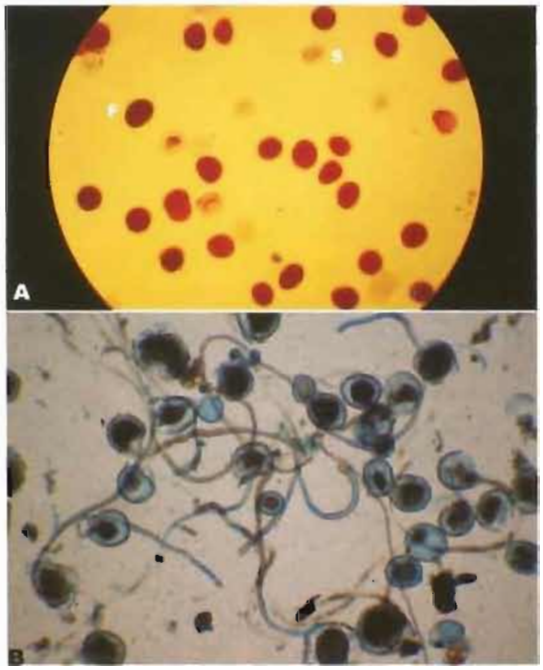
A collection with high pollen fertility was identified from the germplasm of ginger by pollen fertility screening through stainability as well as *in vitro* germination. Ginger Acc. No. 195 showed mean pollen fertility of 67.73% by glycero-carmine staining and 60.31% by *in vitro* germination. Pollen germination was observed on stigmatic surface also on self pollination. The genotype is suitable for future studies on induction of seed set in ginger.

Phenology and dry matter distribution

Ginger varieties *viz.*, Rejatha, Mahima, Himachal, Varada and Maran took on an average 30.9, 25.3, 25.7 and 23.9 days for emergence and 74.8, 46.8, 46.4 and 42.6 days for producing first tiller under 30 April, 15 May, 30 May and 15 June planted crops, respectively. The dry matter distribution (estimated by destructive sampling at fifth month after planting) indicated on an average 12.35%, 65.34%, 7.69% and 14.61% dry matter in root, rhizome, pseudo-stem and leaf, respectively.

Essential oil composition of fresh and dry ginger rhizomes

Comparison of essential oil constituents of fresh and dry ginger rhizomes indicated that fresh rhizomes contained higher level of monoterpenes namely, Z-citral and *E*-citral whereas the dry rhizomes were predominated by the sesquiterpene hydrocarbons namely, zingiberene, farnesene and sesquiphellandrene.



A. Pollen stainability in ginger Coll. No. 195. F-Fertile, S-Sterile

B. Pollen germination in ginger Coll. No. 195

Figure 5. Pollen stainability and germination in ginger Acc. No. 195

Drying methods and quality

Ginger variety Varada was dried with and without peeling in hot air oven, reverse flow and sun dried and evaluated for oil, oleoresin and gingerol contents. No variation was observed in oil and oleoresin contents between peeled and unpeeled samples but unpeeled samples took more time for drying.

SHOOT BORER (*Conogethes punctiferalis*)

Germplasm screening

Four hundred and ninety two accessions of ginger (*Zingiber officinale*) were screened in the field against the shoot borer (*Conogethes punctiferalis*). The accessions were rated for their resistance/susceptibility and 49, 251, 130 and 62 accessions were rated as resistant, moderately resistant, susceptible and highly susceptible, respectively to the pest.

Figure 6. A new hymenopteran parasitoid recorded on ginger shoot borer

Life cycle studies

The life cycle of the shoot borer was studied on 5 resistant accessions (Acc. Nos. 31, 247, 409, 430 and 631), 6 highly susceptible accessions (Acc. Nos. 17, 43, 70, 190, 191 and 514) and one susceptible cultivar (Varada). In the resistant, highly susceptible and susceptible accessions, the pupal period varied from 6-21, 7-11 and 8-9 days respectively. The adult longevity was 2-3, 2-4 and 3 days on resistant, highly susceptible and susceptible accessions respectively.

PAPRIKA AND PAPRIKA LIKE CHILLIES

Germplasm maintenance

Five collections (two from YSPUHF, Solan, Himachal Pradesh, two from Kerala and one from Tamil Nadu) were added to the germplasm. Fifty four germplasm accessions including 21 Bydagi collections and six exotic collections were raised and multiplied. Twenty seven new germplasm accessions were purified by single plant selection and by selfing.

Characterization of germplasm

Morphological and quality characterization of germplasm accessions revealed maximum variability in fruit weight (31.26% CV) followed by yield per plant (30.88% CV). Least variability (3.34% CV) was recorded for pericarp thickness.

Colour

Among the indigenous germplasm accessions, ICBD-11, ICBD-23 and ICBD-10 registered the highest colour value of above 300 ASTA units. In case of exotic lines, high colour value was recorded in EC-71, EC-6, EC-490 and EC-18. The capsaicin content among germplasm accessions varied from 0.0081 to 0.513 %. Overall, the lines ICBD-10, Kt-pl-19 and EC-18 were found promising with high colour value and low pungency.

VANILLA

Flowering in *Vanilla tahitensis*

Vanilla tahitensis, the second commercially important species of vanilla after *V. planifolia* flowered at IISR Farm, Peruvannamuzhi, for the first time, after 4 years of growth. The flowering occurred in two phases unlike in *V. planifolia* planted in the same field. The first phase started in the month of September and ended by October. Second phase stared in the month of February and is in progress.

Occurrence of *Bean common mosaic virus* (BCMV) on vanilla

A 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.

A. Profuse flowering in Vanilla tahitensis
B. Front view of a single flower, enlarged

Figure 7. Flowering in *Vanilla tahitensis*

TREE SPICES

NUTMEG

Evaluation of progenies

In the progeny evaluation trial of elite nutmeg lines, accession A9/185 recorded maximum mean height (592 cm), width (582 cm), number of main branches (63.2) and girth at 30 cm above ground level (50.3 cm), while elite line A9/150 recorded the maximum mean number of fruits per plant (28).

CASSIA

Germplasm registration

The cassia elite line A1 (IC No. 370400) has been registered with NBPGR, New Delhi for

high cinnamaldehyde content in bark oil (81.5%) and leaf oil (80.5%) (Reg. No. INGR 08045).

Morphological and quality evaluation

Significant differences were observed among the 4 elite cassia lines for plant height, width and number of main branches. Elite line D1 recorded the maximum plant height (296.9 cm) and width (234.8 cm) and number of main branches/plant (14.6). Biochemical characters namely bark oil, oleoresin and cinnamaldehyde were recorded for 15 accessions grown at Appangala. Bark oil ranged from 1.2 (D1) to 3.71% (B2) and percentage of oleoresin varied from 7.52 (B8) to 14.14 (B2). Cinnamaldehyde content varied from 65.07 (B8) to 89.63 % (D1). RAPD profiles were developed and species inter relationships was studied in seven species of *Cinnamomum*.

Essential oil constituents of *Cinnamomum* species

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 β-phellandrene, α-phellandrene, camphor, t-*caryophyllene* and *germacrene*-D respectively.

Essential oil from leaves of *C. tamala* was dominated by eugenol whereas the oil from petiole, terminal shoots and shoots contained α-phellandrene and p-cymene as major components. Similarly, t-caryophyllene was the chief compound in the leaf oil of *C. malabatrum* and the essential oils from petiole, terminal shoots and shoot were rich in linalool. The essential oil from petiole and terminal shoots of *C. sulphuratum* contained linalool as chief constituent whereas that from leaves contained β-phellandrene. Essential oils from flowers of *C. verum* and *C. malabatrum* were dominated by t-caryophyllene.

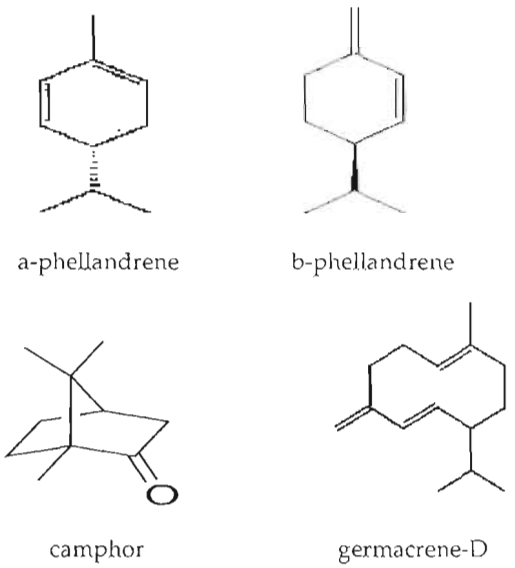


Figure 8. Leaf essential oil constituents of *Cinnamomum* species

SPICES

Detection kits for viruses

ELISA and PCR based diagnostics for the sensitive detection of viruses infecting black pepper and vanilla were developed.

Cross compatibility of *Colletotrichum* isolates infecting spices

Colletotrichum gloeosporioides, the incitant of leaf blight in cardamom survives upto 5 months in simulated field conditions. In order to identify the compatible groups among the isolates, selfing and crossing of predominant *Colletotrichum* isolates infecting black pepper, cardamom and turmeric in all possible combinations was made. The results indicated that, all the isolates were heterothallic (lack of perithecial formation when the isolates were selfed). However, when the isolates were crossed in all possible combinations perithecial formation was not observed in any of the combinations but the crosses resulted either in the formation of a barrage at the point of

contact between two colonies (barrage reaction) or in merging of both the colonies (merging reaction).

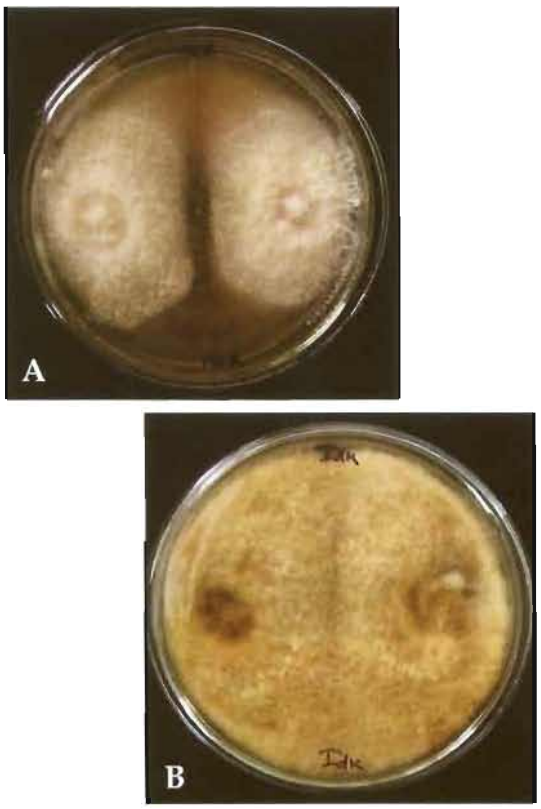


Figure 9. Barriage (A) and Merging (B) reaction of *Colletotrichum* isolates

Characterization *Aspergillus* isolates from spices

Morphological characterization of 15 isolates of *Aspergillus* from pepper, ginger, turmeric and nutmeg has been done using six different mycological media. Among the fifteen isolates, 6 have been identified as toxigenic and nine as non- toxigenic isolates.

Endophytic bacteria from black pepper and ginger

Biosurfactants from different endophytic bacteria were extracted and the compounds were identified as massetolide A using RP-HPLC studies. Among the eight bacteria screened, the highest production of the biosurfactant was noticed in *P. aeruginosa* IISR GB 9 followed by IISR BP 35. *In vitro* and *in vivo* studies using this cyclic lipopeptide showed biocidal activity against *Phytophthora capsici* and *Pythium myriotylum*. Protease enzyme from *P. aeruginosa* IISR 853, extracted and purified by RP-HPLC, possessed high nematicidal activity against *R. similis*.

Isolation and characterization of rhizobacteria from black pepper and ginger

Fifty-five rhizobacterial strains, 24 from black pepper and 31 from ginger were isolated from soil samples collected from Kerala and Karnataka during 2008-09, making the total collection to one hundred and seventy four, 74 from black pepper and 100 from ginger. Four isolates from black pepper and six from ginger were short-listed as efficient based on their *in vitro* antagonistic activity against *Phytophthora*, *Pythium* and *Fusarium*. The identity of these eight isolates was confirmed through 16S rDNA sequence analysis. Three belonged to *Pseudomonas aeruginosa*, two were *Serratia marcescens*, one each belonged to *Klebsiella*, *Bacillus amylolequifaciens* and *Bukholderia pyrrocinia*. The sequences of these isolates were deposited with National Centre for Biotechnology Information databases (NCBI).

Entomopathogenic nematodes from ginger and turmeric

Four strains of entomopathogenic nematodes were isolated from 71 soil samples collected from rhizosphere of ginger and turmeric. Out of these, one strain each was tentatively identified as *Heterorhabditis* sp. and *Steinernema* sp.

Antioxidant potential of spice extracts

The antioxidant property of spice extracts of black pepper (Thevam), ginger (Rejatha), turmeric (Alleppey Supreme) and cinnamon (Nithyasree) were compared at different time

periods and quantified using the *in vitro* methods: total antioxidant capacity by the phosphomolybdenum method, DPPH radical scavenging ability and Fe(III) to Fe(II) reducing activity. DPPH radical scavenging activity was less in water extract while total antioxidant capacity and Fe(III) to Fe(II) reducing activity were greater in water extract compared to the other extracts.

TRAINING & TRANSFER OF TECHNOLOGY

Training programmes

The training programmes conducted during the year were

- Production and processing technology of spices for Department of Horticulture and Food Processing, Uttarakhand, 1-6 May 2008
- Orientation training programme on research and development in spices for field officers of Spices Board, 21-24 May 2008
- Production and processing technology of ginger and turmeric for agricultural assistants, Department of Agriculture, Kerala, Palakkad District, 17-20 August 2008
- Training workshop under the technology mission for integrated development of horticulture in NE states -MM-I at Horticulture Research Complex, Nagecherra, Agartala, 3-5 February 2009

Technology dissemination

Front line demonstration: Front line demonstration programme on performance of improved varieties of black pepper released from IISR was laid out in 18 selected farmers plots in three villages in Calicut District. A multi disciplinary team of scientists visit these plots as per a schedule to follow up and provide advisory services. A total of 14 visits were carried out to these fields.

Video conferencing: Under the Rashtriya Sam Vikas Yojana/VSAT programme sponsored jointly by ISRO and Kerala State Planning Board, 10 video conferencing lessons were broadcast to the 5 Village Resource Centers in Wayanad District of Kerala. Three hundred and nineteen farmers from 5 VRCs attended the sessions.

Impact of technologies developed by IISR

Studies on performance 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.

BIOINFORMATICS

New databases

The Bioinformatics Centre has developed the following databases during the period under report.

- **SpicEST:** A database of ESTs of two major spices, turmeric and ginger was developed and hosted (www.spices.res.in/spicest). SpicEST contains all ESTs of these plants, their annotation, and information on SSRs and SNPs.
- **PASSCOM:** The database on predicted activities of spice compounds was updated

with information on 480 chemical constituents present in seven spices.

- **Spice Genes:** The black pepper germplasm database was updated with several additional features. Another database on cardamom germplasm was developed for the first time.
- **Radobase:** A new database on burrowing nematode containing the information on various *Radopholus* species and their sequence information was developed. About 70 miRNAs were identified in *R. similis* using *in silico* tools.

Figure 10. A view of the SpicEST database

miRNAs in spices

A total of 47 microRNAs were predicted in turmeric, 82 in ginger and 98 in paprika using *in silico* approaches on EST resources. The results were incorporated in SpicEST database. A microRNA blast tool was also developed using NCBI – BLAST stand alone version 2.2.19.

Bioinformatics training

Two Bioinformatics training programmes viz., Agri-Bioinformatics: Tools and Applications, 20-24 October 2008 and *In silico* analysis and annotation of ESTs, 17-19 December 2008 were held during the year for scientists/research fellows.

Figure 11. PASSCOM - database on predicted activities of spices compounds

HUMAN RESOURCE DEVELOPMENT

National Seminar on Piperaceae

Dr D.P. Ray, Vice-Chancellor, Orissa University of Agriculture and Technology, Bhubaneswar inaugurated the National Seminar on Piperaceae, held at IISR, Calicut during 21-22 November 2008. The seminar was organized mainly to discuss the problems faced by farmers in black pepper and betelvine cultivation and to educate farmers on the latest technologies available to improve production. Around 250 delegates including many farmers from different Southern and North Eastern states attended the Seminar.

ICAR sponsored Winter School

IISR organized two ICAR sponsored training programmes during the year.

Winter School on Flavours, Nutraceuticals and Food Colours from Horticultural Crops: This was organized from 7th to 28th January 2009. The Course Director was Dr. B.

Figure 12. Shri Jairam Ramesh, Union Minister of State for Commerce addressing the delegates during the National Seminar on Piperaceae.

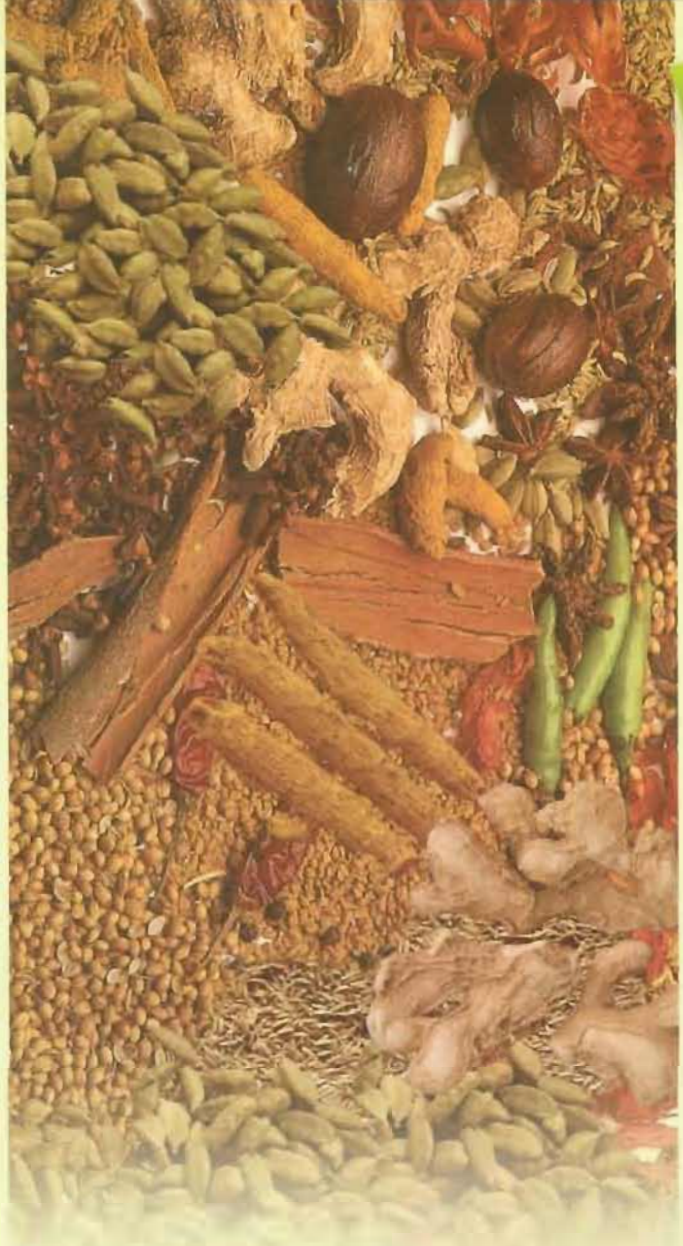
Chempakam, Head, Division of Crop Production & PHT and was co-ordinated by Drs. A. Shamina and K.N. Shiva. The Winter School was inaugurated on 7th January 2009 by Dr. G.R.C. Reddy, Director of NIT, Calicut, and presided over by Dr. V.A. Parthasarathy, Director, who also delivered a special lecture on 'Spicing the flowers'. There were 16 participants. There were lectures (including guest speakers) and practical exercises on flavor and

Figure 13. Inauguration of the Winter School on Flavours, Nutraceuticals and Food Colours from Horticultural Crops

color principles and nutraceuticals present in horticultural crops.

Application of GIS in Plant Biodiversity and Horticulture: This was conducted from 25th February to 6th March 2009. Dr M. Tamil Selvan, Director, DASD, Calicut inaugurated the training programme. The training was attended by 15 participants (5 from ICAR, 6 from universities, 2 from colleges and 2 research fellows). Dr T. John Zachariah and Dr Utpala Parthasarathy co-ordinated the training programme. The most attractive part of the training was hands on sessions on GIS and lectures on GIS application in various fields.

Figure 14. Training programme on Application of GIS in Plant Biodiversity and Horticulture in progress



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