

*Research Highlights*  
**2009-10**



**Indian Institute of Spices Research**  
Calicut



# Research Highlights

2009–10



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

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## PREFACE

The Research Highlights for the year 2009-10 presents the summary of our achievements. The institute enriched its germplasm on all the mandate crops and an alternate germplasm block for conserving the wild *Piper* species were established at CRC, Appangala and CPCRI, Kidu. Microsatellites developed for *Piper* species was successfully used to detect polymorphism in black pepper cultivars. Medicinal plants like Vetiver and Alpinia were found to be profitable as potential intercrops in black pepper. PCR technique is being used to identify virus-free nucleus materials of all released varieties of black pepper for further multiplication in nurseries. Two promising cardamom accessions (IC547146 and IC349630) with high yield and more capsules per plant were shortlisted for further evaluation.

Cross species amplification studies of microsatellite markers were carried out in 13 *Curcuma* species. Partial sequence of *pal* gene was isolated. Targeted application of nutrients based on soil test results increased yield in ginger and var. Mahima was more suitable for organic cultivation. Potential entamopathogenic nematodes were isolated from rhizosphere soils of ginger. Antioxidant potentials of black pepper, ginger, turmeric and cinnamon extracts were studied and no change in the activity potential was noticed on storage. Chemo-profiling of curry leaves essential oil showed no major changes except a decrease in l-phellandrene over storage. *In silico* screening on drugability of spices based phytochemicals was studied.

Impact assessment studies to assess the level of adoption of scientific cultivation practices in black pepper revealed an increased benefit: cost ratio realization with technology adoption by farmers. Our scientists have been able to successfully demonstrate our technologies in black pepper in about 12,000 ha in Kodagu District of Karnataka. The KVK and ATIC conducted programmes suited to the farmers' need and trained more than 4000 beneficiaries. Technology week and Media meet were also conducted for showcasing the technologies developed by the institution.

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 running our programmes. I appreciate the editors for having compiled and brought out this compilation.

Calicut  
05-04-2009



V.A. Parthasarathy  
Director



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## BLACK PEPPER

### CROP IMPROVEMENT

#### Genetic resources

A survey was conducted to collect black pepper accessions (cultivars) in Thodupuzha of Idukki District, Poonjar and Valamukku of Kottayam District, Perumbavoor, Malayattoor and Kunnathunadu Taluk of Ernakulam District. Vettilappara and Athirapally were the places surveyed in Kunnathunadu Taluk. Altogether 105 accessions were collected and planted at Experimental Farm, Peruvannamuzhi after taking proper plant protection measures. A *Piper* species from Dimapur, Nagaland was also collected. In the black pepper germplasm conservatory 2595 accessions are conserved in the nursery and field genebank (Wild pepper- 1286, Cultivars- 1300, Exotic species- 9). In addition to the germplasm established already, 100 more accessions were planted at CPCRI, Kidu. An alternate germplasm block for conserving the wild *Piper* species was established at CRC, Appangala and is being augmented periodically by planting wild accessions. The unidentified *Piper* species collected from North Eastern Region were identified with the help of Central National Herbarium (BSI), Kolkata.

#### Root stock studies

Screening studies helped to identify *Piper ornatum*, a wild species related to black pepper, resistant to *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita* involved in quick and slow wilt of black pepper. Grafting was attempted with var. Sreekara as scion on the species as rootstock. But the growth is poor although the union is strong. Hence, other *Piper* species compatible with black pepper was tested as possible interstock for further testing using *P. ornatum* as rootstock. *P. betle*, *P. longum*, *P. argyrophyllum*, *P. chaba*, *P. attenuatum* and *P. colubrinum* were found to grow satisfactorily and appeared compatible. However, *P. galeatum* failed to grow.

#### Development of core ESTs and cloning of genes

Homology searches for three genes were undertaken and primers were designed. The specific genes include Betaine aldehyde dehydrogenase, Glutathione *S*-transferase and Super-oxide Dismutase for amplification from mRNA of field grown black pepper plants. Transcript based cloning attempts for WRKY regulatory gene resulted in the amplification of a fragment of size of approximately 100bp.

### CROP PRODUCTION

#### Intercropping in black pepper juvenile garden

Intercropping medicinal plants in juvenile black pepper garden recorded a maximum net return of Rs. 46,225 per ha from Black pepper + *Vetiveria zizanioides* followed by Black pepper + *Alpinia calcarata* (Rs. 44,600) with a Benefit: Cost ratio of 2.3 and 2.2, respectively. Height of the plants varied from 129.7 to 154.7 cm and maximum height was recorded in *Ayyapana tripli* intercropping and no reduction in height of plants was observed due to intercropping.

#### Nutrient and plant hormone levels in relation to bearing

Quantification of nutrient and plant hormone levels in the alternate bearers showed that the stem and leaf nutrients levels were low during the bearing year compared to non-bearing year. Carbohydrate to cytokinin ratio was more during the bearing year compared to non-bearing year.

#### Influence of storage on quality

Storage of black pepper powder prepared by hammer mill and manual grinding showed reduction in oil and oleoresin content after five months of storage. Profound reduction was observed in manually ground sample.

Medicinal value of each lot was analysed by examining the antioxidant property using alcohol extract, water extract and petroleum ether extract. Five months storage of pow-

dered sample did not show change in antioxidant property. Black pepper oil constituents were monitored using GC-MS for the five months storage period. Significant change was observed for pinene, myrcene, limonene and linalool.

## CROP PROTECTION

### *Phytophthora* foot rot and slow decline diseases

#### Evaluation of chemicals

Three chemicals such as captan hexaconzole, benzoic acid and salicylic acid were tested *in vitro* and *in vivo* against *P. capsici*. Among them, 100% inhibition of mycelial growth was obtained at 100 ppm in captan hexaconzole and benzoic acid and at 200 ppm in salicylic acid. *In vivo* experiments with these chemicals indicated disease inhibition of 46.2%, 46.2% and 16.4%, respectively.

Four new chemicals *viz.*, Sectin (Fenamidon 10% + Mancozeb 50%), Equation Pro (Famoxadone 16.6% + Cymoxanil 22.1%), Curzate M8 (Cymoxanil 8% + Mancozeb 64%) and Acrobat 50 (Dimethomorph 50%) were evaluated *in vitro* against *P. capsici*. Among the four chemicals tested, Acrobat showed 100% inhibition at 50 ppm concentration. Sectin and Equation Pro showed 100% inhibition of mycelium at 400 ppm and > 500 ppm, respectively. These fungicides are under *in vivo* testing.

Metalxyl sensitivity of *Phytophthora* isolates was tested with different concentrations of Metalaxyl-mancozeb in comparison with Mancozeb. Metalaxyl-mancozeb could inhibit (100% inhibition) the growth of *Phytophthora* isolate at 5 ppm whereas Mancozeb could inhibit the growth at 200 ppm.

#### Evaluation of promising antagonists

Among eighty endophytic fungal isolates isolated from black pepper and evaluated *in vitro* against *P. capsici*, four isolates (EFP 11, 73, 81 and 83) were found promising with >80% inhibitory effect on mycelial growth of *P. capsici*.

The efficacy of endophytic bacteria (BP-35, 25, 17 and TC-10) and rhizobacteria (IISR-853 and IISR-6) against *P. capsici*, *R. similis* and *M. incognita* was evaluated in the field at Peruvannamuzhi. Observations on growth parameters indicated that application of TC-10 + Metalaxyl-mancozeb and IISR- 853 + Metalaxyl-mancozeb were found promising with increased height and canopy of the plant without any disease incidence.

#### Integrated management in the nursery

An experiment was conducted in the nursery for the production of healthy disease-free planting material with four different soil disinfection methods followed by integration of plant protection chemicals and biocontrol agents. Observations on disease incidence (under challenge inoculation conditions) showed that all the disinfection methods and integration of chemical applications such as copper oxychloride (0.2%) + phorate and potassium phosphonate (0.3%) + phorate were promising in reducing *Phytophthora* infection under challenge inoculated conditions.

#### Resistance

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. Selfed progenies of *Piper colubrinum*, a species known to be resistant to *phytophthora* infection, segregated for *phytophthora* resistance.

The activities of cinnamic acid-4-hydrolase (C4H) and caffeic acid-O-methyltransferase (COMT) increased in the susceptible black pepper variety Sreekara than in the resistant hybrid (HP 39) and wounded samples on inoculation with *R. similis*. Lignin content was comparatively low in HP 39 compared to Sreekara. But in HP 39, it increased to double the control values in a month after inoculation with *R. similis*.

#### Establishment of virus-free mother vines

PCR technique was used to identify virus-free nucleus materials of all released varieties of





black pepper. They are being maintained under insect-proof conditions and used for further multiplication in nurseries.

### Transformation of black pepper

Two constructs each of *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV) in *Agrobacterium tumefaciens* EHA 105 were used for transformation. The embryogenic mass was infected with *Agrobacterium* harbouring respective constructs and cultured on basal SH medium for 48 h. The co-cultured embryogenic mass was transferred to selection medium and growing points were removed onto the same medium with a higher kanamycin concentration. The proliferated embryogenic mass was then transferred to basal SH for further development into fully developed plantlets. Fully developed plantlets were transferred individually to WPM. Total genomic DNA isolated from leaves of fully developed plants was used to confirm the presence of inserts through PCR. The PCR positive plants were hardened under green house for challenge inoculation with viruses.

### Screening of germplasm against Pollu beetle

One hundred and eighty five accessions of black pepper were screened against pollu beetle (*Lanka ramakrishnai*). Among the cultivars, Accs. 78 and 4044 were free from pollu beetle attack during the year. The highest berry damage (36.5 %) was recorded on Acc. 1484 followed by Acc. 1611 (34.3 percent). Among hybrids, Accs. 1055, 1069, 1769 and 1752 remained free from pollu beetle damage during the year. Acc. 1339 recorded 100 % berry damage, followed by Acc. 807 (73.5 %).

## CARDAMOM

### CROP IMPROVEMENT

#### Genetic resources

Five accessions were collected from Sabarimala area of Kerala making the total

germplasm repository to 447 (Malabar 278, Mysore 78, Vazhukka 63 and others 28). Morphological characterization has been recorded in 50 accessions. Accessions IC547146 and IC349630 were short listed for high yield with more number of capsules per plant.

### Evaluation of hybrids

Evaluation of  $F_1$  hybrid progenies of Preliminary Evaluation Trial (PET)-I (19 combinations) and PET-II (10 combinations) was carried out and two high yielding selections were short listed for further evaluation. Hybrid GG X NKE – 19 was promising with more than 70 per cent 8 mm capsules and showed field tolerance to rhizome rot and leaf blight. Among the materials shortlisted for Multi Location Trial, NHY-10, MA-18 and SAM performed better compared to others during the year.

### Identification of molecular markers linked to *Katte* resistance genes

DNA was extracted from two parents *viz.*, Green gold (*katte* susceptible) and NKE 12 (*katte* resistant) and PCR was performed with seven sets of ISSR primers (834a, 841a, 854a, 866, 867, 812, and 815) to study parental polymorphism. Two primers (866, 815) have shown polymorphism, primer 866 for susceptible parent-GG and primer 815 for resistant parent-NKE12.



Fig 1. GGx893 cross with good yield and green capsules

## CROP PRODUCTION

### Drought tolerance

Three genotypes RR1 (IC349591), CL-893 (IC 349537), green gold (IC 349550) relatively tolerant to moisture stress and CCS-1 (IC 349589) a susceptible genotype were crossed to develop drought tolerant variety with good yield and quality characters. CL- 893 and its cross combinations recorded better growth and yield parameters which were reduced under stress. Number of tillers per clump ranged from 20-37 with a mean of 29 in control and in stress it ranged from 12.9-32.2 with a mean 20.7. Number of panicle per clump ranged from 20.6-37.4 with a mean of 28.5 and in stress it ranged from 11.4-20.8 with a mean of 16.

### Essential oil profile

Twenty accessions of cardamom germplasm were evaluated for essential oil content. Oil content in capsules ranged from 3.5-6.7% on dry weight basis, with highest in GG-self followed by GG-OP (6.0%). GC-MS analysis of the oil indicated that these accessions contained 26.2% and 27.7% 1,8- cineole and 45.8% and 46.2%  $\alpha$ - terpinyl acetate, respectively.

## CROP PROTECTION

### Screening of germplasm

Thirty eight breeding lines and 20 collections of small cardamom were screened under natural field conditions to identify resistant sources against leaf blight and rhizome rot. Six genotypes each of breeding lines and collections exhibited highly resistant reaction to rhizome rot. Six genotypes *viz.*, 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, respectively.

### Development of diagnostics for viral diseases

Six katte isolates were collected from Karnataka (5 isolates) and Kerala (1 isolate) and established under insect-proof glass house conditions. Enzyme Linked Immunosorbent

Assay (ELISA) based detection of CdMV was standardized and the titre of the antiserum was found to be 1:250. 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. The RT-PCR method was validated by testing more than 50 cardamom field samples representing different geographical locations of Karnataka and Kerala.

Two *kokke kandu* isolates from Karnataka and one Nilgiri Necrosis disease isolate from Suryanelli (Idukki District, Kerala) were collected and established under insect-proof glass house conditions. Surveys conducted in major cardamom growing areas of Karnataka and Kerala, revealed the prevalence of *Banana bract mosaic virus* (BBrMV) infection in Sirsi (Uttara Kannada District, Karnataka), Upputhara, Konnathady and Vellathooval villages (Idukki District, Kerala). The symptoms induced by BBrMV in cardamom included discontinuous or continuous spindle shaped streaks along the veins, continuous light green or light yellow streaks along the midrib and discontinuous mottling along the pseudostem and petiole.

## TURMERIC

## CROP IMPROVEMENT

### Genetic resources

At the field germplasm conservatory, 1173 accessions are being maintained. The breeder's seed of released varieties of turmeric (7 varieties) were multiplied.

### OP seedling progenies

Pot culture evaluation of 256 seedling progenies and 23 mother plants of turmeric showed high variability with respect to yield. Twenty nine progenies showed multiplication rate above 20 times. Of the 68 second generation



seedling progenies of five seedling progenies, many showed very high multiplication rate.

### Self incompatibility studies

Sixty five flowers of Rajendra Sonia and more than 100 flowers of BSR-2 were manually self pollinated but no fruit set was observed. Pollen fertility analysis by staining showed 60.4% fertility in Rajendra Sonia and 55% in BSR-2. *In vivo* germination on stigma was found to be sparse after 4 h of pollination. Operation of self incompatibility mechanism in these varieties is suspected.

### Microsatellite markers and characterization

Cross species amplification studies of microsatellite markers were carried out in 13 *Curcuma* species. Two synonymous *Curcuma* species viz. *C. zedoria* and *C. malabarica* showed identical SSR profiles for 40 microsatellite loci.

## CROP PRODUCTION

### Flowering on yield and dry recovery

The flowering and non-flowering plants of turmeric were compared for yield and dry recovery. On an average, flowered plants recorded fresh yield of 504 g/plant with a range of 298 to 956 g/plant and CV of 41.9%, whereas, non-flowered plants yielded 524 g/plant with a range of 321 to 861 g/plant and

CV of 31.1%. Similarly, mean dry recovery of flowered plants was 15.9% with a range of 10.0 to 19.2% and CV of 21.1%; whereas, non-flowered plants recorded 17.5% with a range of 12.7 to 19.8% and CV of 17.2%. The data was subjected to t-test and it was found that flowered and non-flowered plants did not differ in yield and dry recovery.

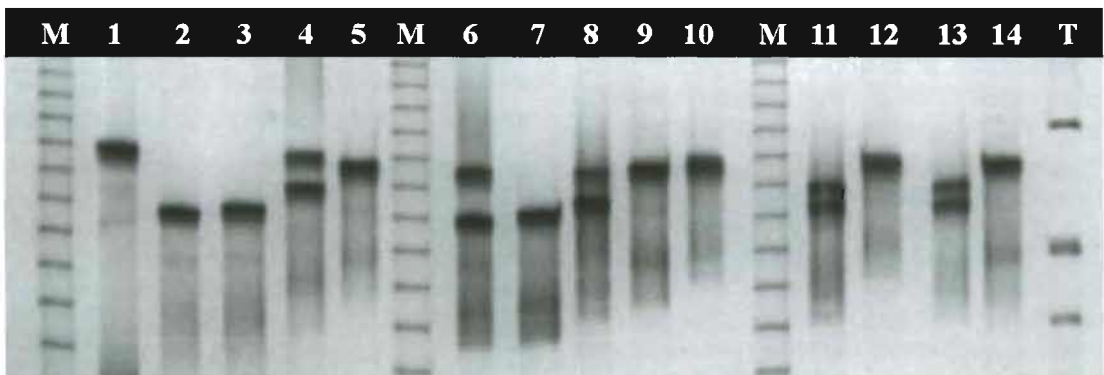
### Cloning of *pal* gene

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. BLAST analysis revealed that the sequence showed 94.3% identity with the *pal* gene from ginger.

## CROP PROTECTION

### Evaluation of chemicals against rhizome rot

The field trial at Settiputhur (Coimbatore District) to evaluate the efficacy of 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% and carbendazim 0.5% and biocontrol agents



**Fig 2.** Legend (A representative gel picture showing the amplification profile of a genomic SSR marker in *Curcuma* species. Lane M-10 bp DNA ladder, Lane 1 - *C. aeruginosa*, Lane 2 - *C. amada*, Lane 3 - *C. aromatica*, Lane 4 - *C. caesia*, Lane 5 - *C. comosa*, Lane 6 - *C. ecalcarata*, Lane 7 - *C. haritha*, Lane 8 - *C. longa*, Lane 9 - *C. malabarica*, Lane 10 - *C. montana*, Lane 11 - *C. pseudomontana*, Lane 12 - *C. raktakanta*, Lane 13 - *C. sylvatica* and Lane 14 - *C. zedoaria*, Lane T- 20 bp DNA ladder.)

(CLT 102 and CLT 110) for the management of rhizome rot disease indicated that application of metalaxyl-mancozeb 0.125% + CLT110 was at par with individual application of either metalaxyl-mancozeb 0.125% or CLT 110 with a disease reduction of 69.1% compared to 69.7% in individual treatments. There was an increase in yield of 12% in the combined treatment when compared to 13.4% in individual treatments.

### Bionomics of shoot borer and its management

The life cycles of shoot borer, *Conogethes punctiferalis* were studied on five moderately field resistant and five susceptible accessions. The average adult longevity, pupal period and fifth instar larval period were 2.9, 12.4 and 7.5 days, respectively on moderately resistant accessions and 2.8, 15.7 and 7.8 days, respectively, on susceptible accessions. However, the differences were not statistically significant. The trials indicated that among the various insecticides, lambda cyhalothrin 0.0125% was more promising in reducing the percentage of shoots infested by the shoot borer.

## GINGER

### CROP IMPROVEMENT

#### Genetic resources

Six hundred accessions of ginger are being maintained at the field germplasm conservatory. The breeder's seed of released varieties of ginger (3 varieties) were multiplied. Cross specific amplification of rice microsatellites was successfully done in ginger. Three of the primers tested gave good amplification products

#### Yield evaluation of high oil type and exotic collections

Among 13 genotypes analysed for biochemical parameters, oil content varied from 1.2 to 2.8%, the maximum in Acc.162 followed by Acc.95, while the oleoresin content ranged

from 2.0 to 4.6% and fibre from 1.87 to 5.13. Of the 12 exotic (Nepal) lines, the oil content ranged from 1.2 to 2.4%, oleoresin from 3.3 to 4.8% and fibre from 2.50 to 5.11.

#### Yield evaluation of low fiber lines

Eight ginger accessions were evaluated for their biochemical parameters, of which the oil content ranged from 1.2 to 2.0%, while the oleoresin from 1.8 to 3.6%. The fibre content varied from 3.54 to 5.15%, the lowest being with Acc.239 followed by Acc. 91 (4.0%).

#### Polyploidy collection with high pollen fertility

Chromosome number of ginger collection No.195 which was identified during last year as high pollen fertile line was verified from mitotic metaphase plates at the root tips. Analysis of mitotic metaphase plates showed that the plant is a tetraploid having  $2n=44$  in majority of the cells. Few cells with variation in chromosome number were also observed.

### CROP PRODUCTION

#### Nutrient requirement for targeted yield

Based on the initial fertility levels of N, P, K the fertilizer doses for obtaining 25, 35 and 45 kg/6 m<sup>2</sup> bed yield targets in ginger were worked out and applied in 3-5 splits at Appangala on Var. Rio-de-genero. The yield levels observed were 21.7, 28.2 and 35.8 kg/ 6 m<sup>2</sup> bed for the targets 25, 35 and 45 kg, respectively. The highest yield of 35.8 kg/bed was

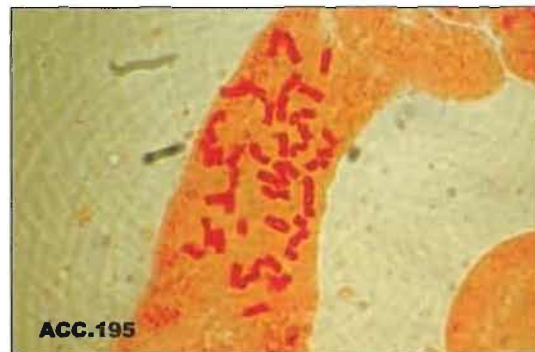


Fig 3. Mitotic metaphase from root tip of Acc. 195





obtained for the target 45 kg/bed. A mean deviation of -13, -19 and -20% at 25, 35 and 45 kg target levels, respectively was observed.

### Organic management on soil quality and yield

Under different management systems like conventional, integrated and organic, the soil nutrient availability of N and K were higher in integrated system which was on par with that of organic system. The Ca and Mg availability was higher under organic system.

Chemical fertilization significantly increased microbial biomass-N, urease activity and metabolic quotient, while organic manuring significantly increased biomass-C, -P, soil respiration, dehydrogenase, acid phosphatase and  $\beta$ -glucosidase activities. This indicated that the nutrient management regimes affected these parameters differently due to changes in microenvironment for microbes, organic C input and nutrient availability across treatments.

The mean yield recorded was 8.1 kg/ 3 m<sup>2</sup> under organic system which was on par to other management systems (7.4-7.7 kg/bed). Under organic management, var. Mahima yielded higher (9.5 kg/bed) and Rejatha yielded on par with conventional system. Highest yield in Varada was recorded in integrated (7.4 kg/bed) followed by conventional and organic systems.

## CROP PROTECTION

### Evaluation of rhizobacterial strains against rhizome rot

The promising rhizobacterial isolates were evaluated for their biocontrol potential against *Ralstonia solanacearum* and *Pythium aphanidermatum* and growth promotion in green house. The isolates GRB 35 and GRB 36 showed highest disease suppression followed by GRB 70 and GRB 91 against *R. solanacearum*. GRB 35 and GRB 91 followed by GRB 68 showed highest disease suppression against

*P. aphanidermatum*. The rhizobacteria were also evaluated in field and the isolate, GRB 68 showed good germination percentage and disease suppression followed by GRB 35 and GRB 70.

Soil biochemical and microbial indices as influenced by rhizobacteria were evaluated in a pot culture experiment. The isolates GRB 25, GRB 36, GRB 38 and GRB 70 applied alone, did not significantly influence microbial activity. However, when applied in combination with 75% N + 100% P + 100% K, gave significantly higher microbial biomass C values. The activities of urease, acid phosphatase, alkaline phosphatase and dehydrogenase were highest in treatments involving the isolates GRB 36 and GRB 38 applied in combination with 75% N + 100% P + 100% K.

### Bionomics of shoot borer and its management

The life cycle of shoot borer, *Conogethes punctiferalis* was studied on five moderately field resistant and five susceptible accessions. The average adult longevity, pupal period and fifth instar larval period were 2.9, 12.3 and 6.4 days, respectively on moderately resistant accessions and 3.0, 11.3 and 6.8 days, respectively, on susceptible accessions. However, the differences were not statistically significant. Among various insecticides, lamda cyhalothrin 0.0125% was more promising in reducing the percentage of shoots infested by the shoot borer.

### Entomopathogenic nematodes

Sixty two rhizosphere soil samples were collected from different locations of Wayanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Guwahati (Assam) and Barapani (Meghalaya) and baited using *Galleria mellonella* larvae. Out of 62 soil samples baited, 6 strains of entomopathogenic nematodes were recorded. Four hundred and twenty four shoot borer larvae were collected from various sites in Kozhikode, Wayanad and Kodagu



districts among which eight were associated with rhabditids nematodes and one with EPN.

## NUTMEG

### CROP IMPROVEMENT

#### Induction of orthotropic shoots in plagiotropic grafts

The auxins and cytokinins in the leaves and buds of orthotropic and plagiotropic shoots of nutmeg were studied and it was observed that the auxin content was highest in the axillary buds of the plagiotropic shoots, whereas the terminal buds contained more auxins in orthotropic shoots. Cytokinin was high in the leaves of both orthotropic and plagiotropic shoots when compared to the buds.

#### Quality

Twenty accessions of mace were analysed for oil content. The oil content ranged from 12-33%, with highest oil yield in IC-548917 (33%) followed by IC- 548922 and IC-548932 (30% each).

## CASSIA

### Yield evaluation

In the clonal evaluation of elite lines at Peruvannamuzhi, D1 (IC 370423) recorded significantly higher height (320.6 cm) and dry bark yield (92 g). Among the 15 cassia lines tested at CRC Appangala, C1 (IC 370415) recorded significantly higher height (545cm), while no significant difference was observed among the lines for number of branches, girth, leaf length and breadth. IC370406 recorded the maximum fresh (1290 g/tree) and dry weight (405.3 g/tree) followed by IC370425 and IC370427. But the highest dry recovery of 56.25% was recorded in IC370401 at Appangala condition on first coppicing. IC 370410, 370423 and 370425 recorded maximum oil yield of 4%. IC370415 recorded 21.57 % oleoresin.

## CINNAMON

Cinnamon bark was extracted in the month of November and powdered in hammer mill for oil and oleoresin. Hammer mill ground sample was stored in three layered polyester cover. Samples were drawn at monthly intervals and analysed for oil, oleoresin and antioxidant activity. Except for slight reduction in oil content no profound change was noted for antioxidant activity due to storage.

## GARCINIA

### Diversity exploration

With the help of BIO CLIM models (Altitude and Rainfall) of DIVA GIS the existence of *Garcinia* in the N E Himalayan states were 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 and Assam.

### Natural food colors and pigments

The fruits or rinds of *G. gummi-gutta*, *G. hombroniana*, *G. indica* and *G. tinctoria* were

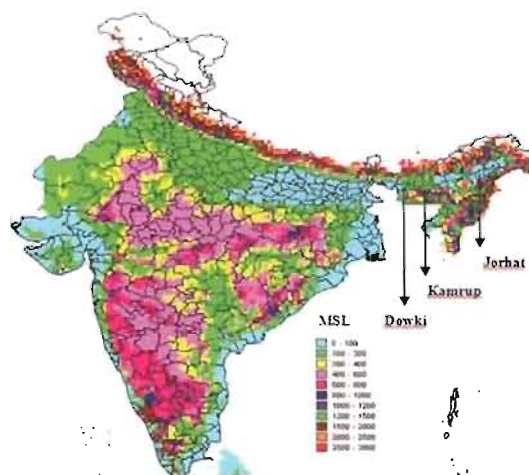


Fig 4. BIO CLIM (Altitude) DIVA GIS model on *Garcinia* diversity



collected and used for color extraction. The total carotenoids content varied from 0.013 in *G. gummi-gutta* to 0.065 mg/100g in *G. tinctoria* and anthocyanin content from 0.007 in *G. hombroniana* to 0.053 mg/100g in *G. indica*. The lycopene content ranged from 3.72 in *G. tinctoria* to 4.95 mg/100g in *G. indica*.

## VANILLA

### CROP IMPROVEMENT

#### Interspecific hybridization

Reciprocal crosses were conducted between *V. planifolia* and *V. tahitensis* (species reported as resistant to root rot disease). High percent of fruit set was observed in both the crosses. Fifty five cultures of selfed progenies of *V. tahitensis* and 60 cultures of *V. planifolia* X *V. tahitensis* were established *in vitro*.



Fig 5. Progenies from (a) *Vanilla tahitensis* (b) *V. planifolia* x *V. tahitensis* grown *in vitro*

## ANTIOXIDANT PROPERTIES OF SPICE EXTRACTS

#### Total phenols

No significant difference was observed in total phenols in essential oils of curry leaves, water and ethanol extracts of black pepper,

ginger, turmeric and cinnamon in the first three months after extraction. But by the sixth month, it reduced to a hundredth of the original value in essential oils and in the water and ethanol extracts it reduced by half.

#### Antioxidant activity

In curry leaves, the DPPH radical scavenging activity of essential oil was reduced by half by the third month of extraction compared to the fresh samples and by another 50% by the next quarter. The water and ethanol extracts were relatively stable in this property over this period. No significant change in total antioxidant capacity, as measured by the phosphomolybdenum method, or Fe(III) to Fe(II) reducing activity was observed in any of the extracts in six months after extraction compared to the fresh samples. Chemo-profiling of curry leaves essential oil showed no major changes except a 50% decrease in 1-phellandrene by the third month after extraction

## OUTREACH PROGRAMMES

### PHYTOFURA

#### Phytophthora characterization

*Phytophthora* isolates (241 isolates) maintained in the repository have been revived and was enriched with 43 new *Phytophthora* isolates collected from different parts of Kerala and Coorg District of Karnataka, bringing the total to 284. The virulence 100 *Phytophthora* isolates were tested *in vitro* by detached leaf inoculation method and grouped the isolates into three *viz.*, highly virulent, virulent and less virulent.

Genomic DNA was isolated from 126 black pepper *Phytophthora* isolates and SSR profiling was done with one primer pair GTCTGCGCTGTCGGA ACT (113 F)/ TRATGATGCGGTTCA TCTCG (114 R). ITS

region of *R. similis* was amplified with universal primers. Species specific markers for detection and quantification of *P. capsici* and *R. similis* have been designed and employed.

ESTs (56,457 numbers) of *Phytophthora capsici* available from dbEST of NCBI were downloaded, cleaned and assembled into 5966 contigs using CAP 3 program. Functional annotation of these contigs revealed that 84.73% of the ESTs displayed significant similarity to known sequences in GenBank. About 3.57% (213 numbers) ESTs were assigned to hypothetical proteins of unknown function while 699 (11.7%) had "no hit". About 223 microsatellites were detected in EST sequences of *P. capsici* and primers were designed for them. A 250 bp fragment was amplified from *P. capsici* genomic DNA.

**PhytoWeb**, a comprehensive portal on *Phytophthora* diseases of Horticultural crops in India was developed by modifying the existing PhyDisH. Phytolib, an electronic database of research publications on *Phytophthora* has also been developed and launched through this portal.



Fig 6. Phytoweb a portal on *Phytophthora* diseases

## LEAF SPOT DISEASES

Surveys were carried out in Karnataka, Kerala and Tamil Nadu for collection of samples of leaf spot diseases. Out of 150 samples includ-

ing cardamom, black pepper, arecanut, turmeric, ginger, chilli and clove, 100 were infected with *Colletotrichum* spp. The cultures were identified based on colony and conidial morphology. The identified cultures were subsequently pure cultured and maintained for further studies. The cultures were further characterized based on colony characteristics and conidial and appressorial morphology.

## BIOINFORMATICS

### *In silico* research

EST assembly and annotation of two entomopathogenic nematodes, *Steinernema feltiae* (83 nos.) and *Heterorhabditis bacteriophora* (53614 nos.) were attempted. Syntenic relationship between *Radopholus similis* and other nematodes was studied using mitochondrial genome sequences.

A virtual screening of phytochemicals from Dr. Duke's phytochemical and Ethnobotanical database, literature search, PASS prediction and ADME/Tox screening has identified 56 promising lead compounds with potential nematicidal activity. Flexible docking studies revealed that six of these phytochemicals - curcumin, brucine-n-oxide, colubrine, brucine, vanillin, genostyrychnine and strychnine, had good binding score (MolDock score) and favorable hydrogen bond interaction with glutathione-S-transferase (GST) of *Brugia malayi*. Thus these phytochemicals can be taken as

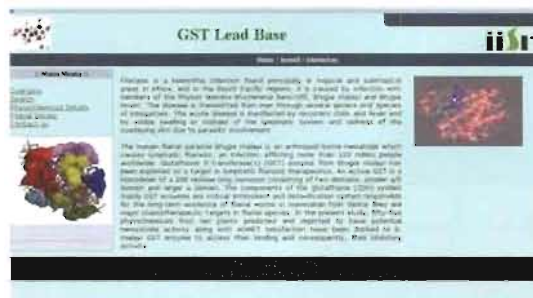


Fig 7. GST Lead Base - data base on potential nematicidal compounds





promising lead compounds to treat filariasis caused by *B. malayi*. *In vitro* assay confirmed that the phytochemicals with the greatest inhibitory potential against canine *Dirofilaria immitis* GST were linalool, alpha-pinene, strychnine, NVA, vanillin, piperine, isoeugenol, curcumin, beta-caryophyllene, cinnamic acid, capsaicin, citronellol and geraniol, in descending order.

### New Database

A new database, GST Lead Base ([www.spicebioinfo.res.in/gstleadbase](http://www.spicebioinfo.res.in/gstleadbase)), was developed and hosted. The database includes chemical properties and other details of potential nematicidal compounds that inhibit glutathione-S-transferase in nematodes.

### IT initiatives

- ❖ *SpiceStat*, the database on vital statistics of spice cultivation, production and export was relaunched with additional features.
- ❖ A new *accounts module* was developed and integrated in ARISoft, the existing office automation software.
- ❖ An *interactive CD* on ginger and turmeric package of practices was developed.
- ❖ As per the guidelines received from ICAR, the IISR website was modified by using Joomla, an open source CMS software, and it is under trial run.

## TRAINING & IMPACT ASSESSMENT

### Training programmes

During the year, 1111 farmers (241 from within district, 157 from the state and 713 from outside the state) availed farm advisory services from ATIC and 1275 farmers visited the experimental farm, Peruvannamuzhi. About 1050 students visited the institute on study tour. The institute conducted one on campus training programme (5 days) for the state department extension functionaries, sponsored

by the Kerala State Horticulture Mission. Two off campus training programme on black pepper for farmers sponsored by M S Swamintahan Research Foundation, Wayanad were organized in Wyanad District. The institute also participated in one farmers' seminar under the Janasree mission of the Kerala Government, five exhibitions/farmers fairs at national level and four at regional level. Seven video conferencing sessions with village resource centers in Wayanad District were organized in which 389 farmers participated.

A Media Meet was also organized on 9<sup>th</sup> November 2009 to mobilize mass media support for sharing Agro-Information, under NAIP project. Over 30 media persons from around 20 various media organizations attended. Over 20 news items appeared in various print media, one audio item in AIR-Calicut and news in channels.



Fig. 8. Media meet on showcasing technologies

### Impact assessment of technologies

Survey of 1615 ha of black pepper plantations in Kodagu District (Karnataka) where IISR had technology intervention programmes to assess the level of adoption of scientific cultivation practices revealed the following level of adoption of technology inputs in the purposive samples: foliar spray of Bordeaux mixture - 100%, soil drenching of fungicides - 80%, application of nematicides - 60%, basin irrigation in summer - 70%, application of chemical fertilizers - 20% and application of bioagents - 20%.

The average yield (with an average of 63%

yielding vines) in the purposive sample was 3.24 kg/standard compared to 1.9 kg/standard in the control plots where conventional practices were followed. The recorded cost of cultivation in the purposive sample was Rs 80 per vine and the estimated cost: benefit ratio at the given level of adoption of technologies was 3.025. In conventional black pepper growing tracts of Kerala, a previous survey recorded a cost benefit ratio of 2.4.

## KRISHI VIGYAN KENDRA

### Training programmes

The KVK has conducted 73 training programmes on various subjects during the period and trained 3024 beneficiaries. About 400 farmers visited during the period for consultation, purchase of planting materials and other inputs from KVK. Three study tours were arranged for the farmers during the period, benefiting 96 farmers. Eight Front Line Demonstrations and four On-farm Trials were undertaken.

### Technology Week / Kisan mela - 'Sankethika Darshanam 2010'

A five day farmer's festival 'Karshika Sankethika Darshanam -2010' was jointly organised by the Indian Institute of Spices Research (IISR), Calicut, Krishi Vigyan Kendra (KVK) and NAIP, at Peruvannamuzhi during 8-12 February 2010. Five award winning



Fig. 9. Inauguration of technology week - Karshika Sankethika Darshanam -2010

farmers were felicitated. Exhibition stalls were put up by six ICAR, Central government and 14 private entrepreneurs and input agencies for showcasing the technologies developed by the institutions. About 250 farmers participated in the technical sessions and about 1500 in the exhibition and benefited by the technological inputs/services provided. Technical sessions on different aspects of agriculture like organic farming, animal husbandry, floriculture, mushroom culture, farm machinery and pisciculture were arranged by inviting technical experts to train and interact with the farmers.



Fig. 10. Technical sessions on different aspects of agriculture during technology week

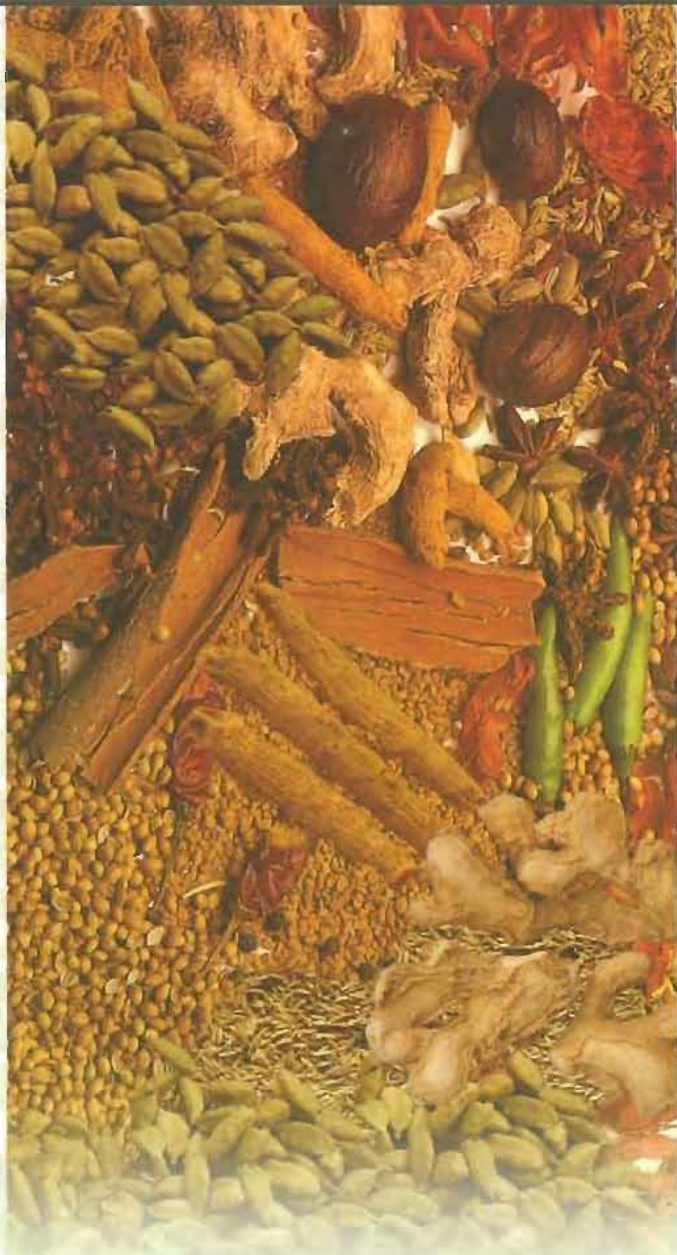
## HUMAN RESOURCE DEVELOPMENT

### Trainings

- ❖ A bioinformatics training programme on 'Recent advances in EST analysis and their annotation' was organized during 20-23 Oct. 2009 in which 17 trainees from various institutes/Universities participated.
- ❖ One month summer training on Biochemistry, Biotechnology and Bioinformatics was conducted for M.Sc. students during 6<sup>th</sup> May – 5<sup>th</sup> June 2009.
- ❖ Twenty-one M.Sc. students carried out project work in various disciplines. Five students have undergone Post M.Sc. training, one for M. Phil project work and one student was awarded Ph.D.







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