



*Research Highlights*  
**2010-11**



**Indian Institute of Spices Research**  
Calicut

# Research Highlights

2010-11



भारत अनुप  
ICAR

**Indian Institute of Spices Research**

(Indian Council of Agricultural Research)

Calicut, Kerala, India.

A detailed microscopic image of plant cells, showing various cellular structures and textures. The cells are arranged in a somewhat regular pattern, with some showing distinct cell walls and internal organelles. The overall appearance is that of a cross-section of a plant tissue, possibly a root or stem, showing the intricate details of its cellular composition.

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## PREFACE

The Research Highlights for the year 2010-11 presents the summary of our achievements. The National Active Germplasm (NAGs) on all the mandate crops have been enriched with additions from centers of AICRP on Spices and NBPGR. Hybrid progeny blocks for pollu resistance and high caryophyllene crosses of black pepper has been established. The causal agent of 'kokke kandu' of cardamom has been identified as a *Nano virus* with closest resembling group as *Banana bract mosaic virus* (BBrMV). Two cardamom accessions (IC-349613, IC-349588) that are highly resistant to leaf blight was identified. A machine for mixing and vending potting mixture was fabricated in collaboration with CIAE, Coimbatore.

In turmeric, soil application of zinc @ 5 kg/ha or as two foliar sprays @ 0.25% has increased the rhizome yield and curcumin. Improved processing method using steam boiler has been standardized. Targeted application of nutrients based on soil test results increased yield by 15-47% over conventional fertilizer recommendation in black pepper and ginger. Potential entamopathogenic nematode strains causing 100% mortality within 72 h on shoot borer larvae were identified. Nutmeg accession A9-71 (INGR10142) is registered with NBPGR for high sabinene (45.0% and 41.9% sabinene in nut and mace oils, respectively).

The impact assessment studies showed that short duration nature of the crop, suitability for early planting in places with assured irrigation, high curcumin content, relative disease tolerance and stable yield over seasons compared to local cultivars are the reasons for the large scale adoption of the IISR varieties by farmers. The KVK and ATIC conducted programmes to the farmers' need and trained more than 6750 beneficiaries. Fifteen audio capsules and three video films were developed on different aspects of spice production technologies. Media visits were organized to farmer's plots and Kisan Mela cum Technology Expo was held at the institute for popularization of technologies.

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 appreciate the editors for having compiled and brought out this compilation.

Calicut

Date : 11.04.2011



V.A. Parthasarathy

Director

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## BLACK PEPPER

### CROP IMPROVEMENT

#### Genetic resources

One hundred and three accessions were collected from the coastal regions of three major thaluks in Alleppey district viz., Karthikapally, Ambalapuzha and Cherthala. The altitude of collection sites ranged from -2 m (below sea level) to 7 m MSL. The total number of collections that are being conserved is 2695 (Wild pepper- 1286, Cultivars- 1400, Exotic species- 9). About 100 accessions were planted at the alternate germplasm center at CRC Appangala. Two hundred hybrid progenies of the pollu resistant cross combination (Subhakara x Coll.816) were planted at Chelavoor for evaluation. Two hundred hybrid progenies of the high caryophyllene cross (Subhakara x Coll.1089) were also planted at Chelavoor.

#### Developing ISSR profiles for identification of released varieties

ISSR profiles were developed for 16 varieties of black pepper with 14 primers, for developing standard profiles and varietal identification. Varietal specific bands were also observed for a few varieties. Identification of varietal specific bands for all released varieties of black pepper is in progress for diagnostics.

#### Functional annotation of EST sequence data from the transcriptome of *Piper*

Assembly and functional annotation of sequences derived from the transcriptome of *Piper colubrinum* and *P. nigrum* helped in the identification of chalcone isomerase, chalcone synthase, cinnamate 4-hydroxylase, cinnamoyl-CoA reductase, geranyl geranyl pyrophosphate synthase, hmg-CoA reductase, lycopene  $\beta$  cyclase, phenylalanine ammonia lyase, p-coumaroyl shikimate 3'-hydroxylase and transaldolase genes involved in secondary metabolism.

#### Cloning of resistance genes- using degenerate primers from NBS motifs of R genes

Degenerate primers approach was used to amplify fragments related to R genes in black pepper. Moderately resistant and susceptible black pepper varieties and *Piper* species were amplified using eight pairs of degenerate primers (64 combinations) designed from conserved NBS- LRR regions of six *Phytophthora* resistance genes from *Capsicum annum*, *C. chinense*, *Nicotiana tabacum*, *Solanum demissum* and *S. tuberosum*. Cloning and sequencing of 520 bp product showed only 50% similarity to NBS-LRR type disease resistance protein. Moderately resistant varieties of Black pepper P24-O-4 had 57% identity to *Solanum trilobatum* NBS-LRR and ISSR Shakthi had 48% identity to disease resistance protein *Brassica rapa subsp. pekinensis*.

#### Host resistance- Screening mapping population for *Phytophthora* resistance

Fifty two lines selected as association mapping population were screened using leaf, stem and root inoculation methods. Accession Kumbachola (Acc. No. 1114) was found to be tolerant to *Phytophthora* infection (Fig 1). This accession was also earlier found to be resistant to Pollu beetle and drought. Seedlings of *P. colubrinum* (178 nos.) on screening with *P. capsici* showed segregation of the resistance character, 21 plants being resistant to *Phytophthora*, 2 plants susceptible and the rest showing moderate resistance.



Fig 1. Stem and leaf screening of Acc. No.1114 (Kumbachola) showing moderately resistant reaction

## CROP PRODUCTION

### Effect of light intensity

Studies on the influence of light intensity on black pepper yield revealed that vines that received light intensity of around 500 to 800  $\mu$  moles  $m^{-2}$   $sec^{-1}$  produced maximum yield (5 kg/vine). Vines which received 200-300  $\mu$  moles  $m^{-2}$   $sec^{-1}$  produced 3.4 kg/vine and those which received around 100  $\mu$  moles  $m^{-2}$   $sec^{-1}$  produced 1.8 kg/vine under Madikeri conditions.

### Nutrient requirement for targeted yield

Based on the initial fertility levels of N, P, K the fertilizer doses for obtaining 5, 7.5 and 10 kg/standard yield targets were worked out and applied at Mirigarajendra estate, Madikeri on Panniyur-I. The recorded yield levels were 7.0, 8.7 and 9.7 kg/standard in the targets of 5, 7.5 and 10 kg/standard, with a deviation of +40.2, +15.6 and -2.9 respectively. Through targeted equation, nutrient requirement for all the yield targets could be predicted with a minimum deviation. Through targeted nutrient supply 6.3-47.3% yield increase as compared to the normal recommendation was realized.

### Machine for mixing and vending potting mixture

A machine was fabricated in collaboration with CIAE, Coimbatore centre which is capable of mixing, pulverizing, sieving, and filling of potting ingredients in poly bags at desired quantity. The unit consists of 3 HP motor, feed hopper, paddles, sieving tray, and electronic vending instrumentation (Fig 2).



**Fig 2.** Machine for mixing and vending potting mixture

For spices nursery, the optimized ratio of soil, granite powder and farm yard manure of 2:1:1 (v/v) was used for preparation of potting mixture in the

machine. Machine can fill around 1600 bags in a day as compared to 600 bags manually by two persons.

## CROP PROTECTION

### Phytophthora characterization

Twenty one new collections of *Phytophthora* were made during the year, bringing the total collections in the National Repository of *Phytophthora* to 305. Seventy *Phytophthora* isolates were characterized for their colony morphology, while 42 were characterized for their sporangial morphology and 37 for their pathogenicity. In black pepper, out of the 137 isolates studied for virulence, 110 were highly virulent and 10 were non-virulent. A1 mating type dominated (66.3%) among the 86 collections studied. Metalaxyl-MZ sensitivity of 81 isolates was tested and the  $EC_{50}$  and  $EC_{90}$  values ranged from 0.0002 to 14.4 ppm and 1.1-68.5 ppm, respectively.

### Genetic diversity and fingerprinting of *P. capsici*

Genetic diversity of 126 black pepper isolates of *Phytophthora* was studied using 20 SSR markers and dendrogram was prepared. The dendrogram of diversity indicated high diversity among isolates and there were 5 major groups further divided into 40 minor groups. This is the first study of diversity among black pepper isolates of *P. capsici* using SSR markers.

The black pepper isolates of *P. capsici* were screened with *P. capsici* specific primer PcapF- PcapR. Expected amplicon of 573 bp was obtained with all the isolates.

### Genomics

In a bioinformatics mediated approach, a set of three primers were designed and out of the three primers, one set of primer ELICPHYF6 and ELICPHYR6 yielded a product of 250 bp, the sequence of which perfectly matched with alpha elicitor of *P. capsici* from the database. Targeted cloning of WRKY transcription factor genes from *Piper colubrinum* yielded a 143 bp gene fragment similar to WRKY sequences already identified in different plant



species. Preliminary transcriptome analysis of transcripts expressed within leaf tissues challenged with *Phytophthora* revealed expression of many stress induced genes as well as genes related to secondary metabolism. A variety of transcription factors, stress induced genes as well as genes related to secondary metabolism with significant similarity to those characterized in other plants were also identified.

### Evaluation of biocontrol agents

Forty five isolates of endophytic fungi were isolated from black pepper making the total isolates available to 125. Out of the 45 isolates tested *in vitro* against *P. capsici*, nine showed more than 70% inhibition. Among the three mycological media tested for the isolation of endophytes, Malt Extract Agar (MEA) was the best medium. Fifteen *Trichoderma* isolates from various locations were tested *in vitro* against *P. capsici* and all the isolates except PhytoFuRa 7 showed >50% inhibition against the pathogen.

The efficient antagonistic isolates of rhizobacteria obtained from black pepper (BRB 3, BRB 13, and BRB 49) and ginger (GRB 35, GRB 68 and GRB 70) were characterized to decipher its mode(s) of action on *P. capsici*. Acetone + EtoAc extractions were obtained and evaluated against the pathogen among which the antibiotic obtained from GRB 68 was promising.

The suitability of a combination of biocontrol agents and biofertilizers in vermicompost was tested for their effect on plant growth and disease suppression in black pepper in the greenhouse. The results showed that a consortium of IISR 853 (*Pseudomonas aeruginosa*) + *Trichoderma harzianum* + biofertilizers was more promising in increasing the growth of plants. However, the lowest (5.6%) disease incidence was observed with IISR 6 (*P. fluorescens*) + biofertilizers. Application of endophytic biocontrol agents TC 10 (*Curtobacterium luteum*) + Metalaxyl-Mz and BP 17 (*Bacillus megaterium*) + Phorate was promising in the field against *P. capsici*, *Radopholus similis* and *Meloidogyne incognita*, recording higher yield. The yield obtained in the treatment TC 10 + Metalaxyl-

Mz was 6.498 kg/vine (fresh) when compared to 1.930 kg in chemical control. No incidence of *Phytophthora* foot rot or slow wilt could be noticed in any of the treatments during the period.

### Viral Diseases

#### Identification of transformants carrying *Piper yellow mottle virus* (PYMoV)

All the hardened plants obtained from both sense and anti-sense constructs were subjected to PCR, dot blot and southern hybridization to confirm the transgene presence. In the case of sense construct, all the 30 plants screened were positive in PCR whereas in anti-sense construct, out of 78 plants screened, 62 plants tested as positive in PCR. The selected PCR positive plants were subjected to dot blot assay which showed that out of 30 plants, 24 were positive in sense construct, and out of 44 plants in antisense construct screened, 38 were positive. In southern hybridization analysis, in the case of sense construct, two plants showed positive signals (out of 10 screened) whereas four out of seven tested were positive in the case of antisense construct.

#### Identification of transformants carrying *Cucumber mosaic virus coat protein* (CMV-CP)

Out of 109 plants screened, 104 plants were positive in PCR test. Out of 80 plants subjected to dot blot, 43 plants gave positive signals and all the nine plants tested were positive in southern hybridization.

#### Challenge inoculation of transgenic plants with PYMoV

Transgenic plants obtained with both sense and antisense constructs of PYMoV were challenge inoculated with PYMoV using mealy bug (*Ferrisia virgata*) as vector. After 60 days of inoculation, the plants were screened for the presence of PYMoV through PCR. The results showed that of the 24 plants carrying PYMoV sense construct challenge inoculated, 12 plants showed positive reaction in PCR while 12 plants tested negative in PCR. In PYMoV antisense construct, of the 60 plants challenge inoculated, 45 were positive for virus in PCR while 15 plants were negative in PCR.



## Secretome analysis

The presence of  $\beta$ -1, 4 endoglucanase, a major secretory cellulose enzyme in nematodes, was located in *R. similis* through EST analysis. Docking studies were carried out using 10 phytochemicals and 17 bacterial metabolites against the target protein  $\beta$ -1, 4 endoglucanase and a few chemicals with least dock score were short-listed. An *in vitro* bioassay using five phenolic compounds namely, cinnamic acid, coumaric acid, ferulic acid, caffeic acid and NVA (*N*-vanillylnonanamide) at four concentrations was conducted with *R. similis* as the test organism. Among these, maximum mortality was observed with the highest concentrations of ferulic acid (70.67%) followed by coumaric acid (65%) and caffeic acid

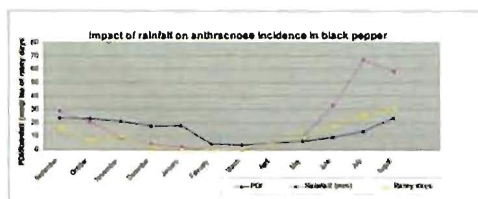


Fig 3. Relationship between rainfall and incidence of anthracnose in black pepper

(48%). The mortality of nematodes was directly proportional to the concentration of the compounds.

## Evaluation of antagonists

Crude culture filtrates from six bacteria (GRB 35 - *Bacillus amyloliquefaciens*, GRB 68-*Serratia marcescens*, GRB 70-*Enterobacter dissolvens*, BRB 3-*Micrococcus* sp., BRB 13-Unidentified and BRB 49-*Serratia* sp.) were tested *in vitro* for their nematocidal activity. Culture filtrates of BRB 13 at 40  $\mu$ l/ml caused 100% mortality of *R. similis* within 24 h.

## Anthracnose - Characterization

Among the *Colletotrichum gloeosporioides* isolates infecting black pepper, BP 10, BP 15, BP 22, BP 24 and BP 26 were identified as aggressive isolates. The aggressive isolates of *C. gloeosporioides* infecting black pepper were inoculated on 12 cultivars/varieties of black pepper to study the differential reaction and

to develop a set of differentials based on the symptomatology. Variability in the symptoms was characterized based on the prominence of the yellow halo around the spot and the diameter of the spot.

The existence of fungicide sensitive or resistant isolates among the field populations of *C. gloeosporioides* infecting black pepper was noticed in Pollibetta and the isolate from this locality was tolerant to recommended doses of Bordeaux mixture and carbendazim.

## Epidemiology

Daily maximum and minimum temperatures had negative correlation with anthracnose disease incidence while rainfall and number of rainy days had positive correlation (Fig 3). The disease gets initiated during May - June in most of the vines and the incidence attains its maximum during August. The disease initiates as small round necrotic lesions on the young leaves of the runner shoots from which it advances to the leaves of orthotrophic and plagiotrophic branches as well as to spikes which resulted in shedding of leaves and spikes.

## Evaluation of biocontrol agents

Thirty two *Trichoderma* spp. were isolated from soil samples collected from rhizosphere of cardamom and black pepper from Appangala (Karnataka), Idukki, Wayanad (Kerala) and Valparai (Tamil Nadu). Among the isolates WYD T11, the *Trichoderma* sp. obtained from rhizosphere of black pepper was identified as a promising isolate. The culture filtrate obtained from the isolate adversely affected conidial germination, appressorial formation and melanization of *C. gloeosporioides*. The isolate was compatible with metalaxyl + mancozeb (at the recommended dose) commonly used in black pepper plantations.

## Evaluation of plant extracts

Extracts (2.5%, 5%, 10% and 20%) of 35 locally available plant species were evaluated against *C. gloeosporioides* (cardamom and black pepper), by employing poisoned food technique, in laboratory bioassays among which extracts of *Solanum nigrum*, *S. torvum* and *Azadirachta indica* were promising.



Hyphae of the targeted pathogen also exhibited modifications like abnormal hyphal branching, hyphal tip swelling and vacuolization.

### Evaluation of fungicides

Among the nine fungicides evaluated for their efficacy against *C. gloeosporioides* infecting cardamom and black pepper *in vitro*, hexaconazole and carbendazim + mancozeb were promising against the pathogen.

## CARDAMOM

### CROP IMPROVEMENT

#### Germplasm characterization

Cardamom field gene bank was enriched with 103 new accessions (73 accessions from KAU, Pampadumpara and 30 accessions from RRS, Mudigere) making the total germplasm to 550. Morphological characterization has been completed in 50 accessions. Accession IC547206 and IC584093 were short listed for high yield and more number of capsules per plant.

#### Evaluation of hybrids

Analysis of 3 successive crop yields of F<sub>1</sub> hybrid progenies of Preliminary Evaluation Trial (PET)-I (19 combinations) and PET-II (10 combinations) resulted in identifying 3 high yielding hybrids such as IC584097, IC584098 and IC54722. These selections yielded 20-40% higher crop than corresponding controls (CCS 1 and Njallani Gold). Among the genotypes evaluated under Multi Location Trial, NHY-10 and MA-18 performed better compared to others.

#### Studies on molecular markers linked to *Katte* resistance

Parental polymorphism of GG (Susceptible) and NKE12 (Resistant) using four SSR primers *viz.*, RM01, RM72, RM117, RM 131 failed to detect polymorphism among the parents. Among the 24 markers, two (866, 815) revealed polymorphic bands each of 350 bp and 250 bp in the susceptible and resistant parents, respec-

tively. Seven primers failed to amplify a clear product while rest gave a monomorphic pattern. Twenty F<sub>2</sub> mapping populations obtained from the cross between GG and NKE 12 were inoculated with aphids for *katte* transmission studies.

#### Morphological characterization

One hundred and ten genotypes of small cardamom depicting maximum diversity including 12 released varieties, 10 farmer's varieties, 4 related genera and 5 unique varieties were selected and a descriptor was prepared with about 46 taxonomically and agronomically important characters based on IPGRI descriptor and DUS guidelines. Data on 40 morphological and floral characters were recorded.

#### Molecular characterization

Restriction ligation, microsatellite enrichment and cloning was completed. Sequencing of 32 cloned products revealed 8 microsatellites. Primer designing is in progress. EST Data base searches for sequence information containing microsatellites from ginger revealed 94 SSR candidates. Twenty primers were designed. Of these seven were found to amplify PCR products in cardamom but only one gave polymorphism between the genotypes.

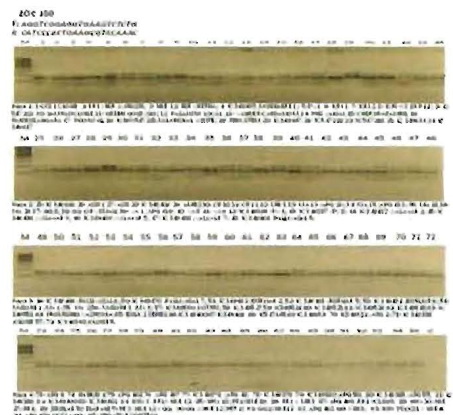


Fig 4. SSR profiling of Cardamom genotypes using Ginger EST SSR marker showing polymorphism

ISSR profiling of 100 small cardamom genotypes were developed using 25 ISSR primers. The dendrogram of inter relationships was prepared. Ginger EST SSRs when tested for small cardamom gave a few polymorphic markers (Fig 4).

## CROP PRODUCTION

### Evaluation of hybrids

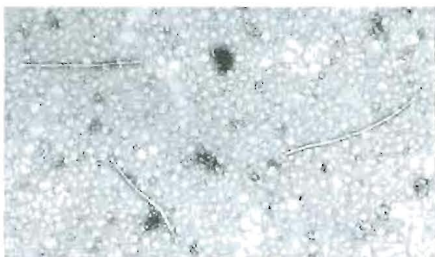
Among the eight cross combinations of cardamom evaluated for essential oil content, with special reference to moisture stress, Bold x (GG x CCS1) and (GG x CCS 1) x Bold recorded higher oil content under stress conditions. Twenty five germplasm accessions were evaluated for essential oil content which ranged from 3.5-6.0%. Acc. 547200 recorded highest oil content followed by Acc. 547201 with 5.5%. Leaf folding test was undertaken to test drought tolerance of cardamom genotypes and (GG x CCS-1) x Bold and Bold x 547219 took longer time to fold.

## CROP PROTECTION

### Viral diseases - Characterization

Based on leaf dip electron microscopy, the causal agent of 'kokke kandu' showed a close resemblance with *Nano virus*. Primers were designed to confirm the presence of *Nano virus* in the samples infected with *kokke kandu* disease. Leaf dip electron microscopy of the samples obtained from symptomatic plants affected with chlorotic streak disease revealed the presence of flexuous virions resembling *Potyvirus* (Fig. 5).

Sequencing and BLAST analysis of the sequence generated with the primer pairs targeting the conserved region of *Potyvirus* (WCJEN) and Poly (A), showed



**Fig. 5.** EM of BBrMV of cardamom

*Banana bract mosaic virus* (BBrMV) as the closest resembling group. Specific primers aimed to amplify coat protein of BBrMV resulted in a product of 950bp size. Sequence analysis of coat protein gene showed an identity of >94% with BBrMV isolates while identity with other distinct potyvirus species were <60%, indicating that causal virus is a strain of BBrMV. A reliable RT-PCR based method was also developed for detection of the virus in plants.

### Distribution

Surveys conducted in major cardamom growing regions of South India, revealed that BBrMV infection in cardamom was prevalent in Karnataka, Kerala and Tamil Nadu and the incidence of the disease ranged from 0 - 15%. The disease was characterized by the appearance of chlorotic streaks along the veins and hence the name 'chlorotic streak' was proposed for the disease.

### Leaf blight - Characterization

The isolates of *Colletotrichum* spp. obtained from infected samples were characterized based on the colour of the colony and five groups were elucidated viz., gray, white, grayish white, grayish olive and pale pink, of which maximum number of isolates were observed in Group IV, grayish olive. Among the *C. gloeosporioides* isolates infecting cardamom, the isolates CD 2, CD 11, CD 25, CD 27 and CD 30 were identified as aggressive.

### Resistance

The natural incidence of leaf blight was recorded in 328 germplasm accessions and two accessions were highly resistant (IC-349613, IC-349588), while 103 resistant, 95 moderately resistant, 91 moderately susceptible, 35 susceptible and 2 highly susceptible.

### Rhizome rot - Distribution

Surveys carried out in Wayanad and Idukki districts of Kerala, Valparai in Tamil Nadu and Hassan and Kodagu districts of Karnataka to study the disease incidence revealed that Meppadi Panchayat in Wayanad district is a hot spot. Forty two fungi were isolated from the 65 samples of rhizome and root rot (Fig 6 a&b) disease collected from different locations during the survey. The fungi included *Rhizoctonia solani*, *Fusarium*



*oxysporum*, *Fusarium solani*, *Fusarium* spp, *Colletotrichum* spp., *Pythium vexans*, *Botryodiplodia theobromae* and six unidentified cultures. Soils samples were collected from the rhizosphere of healthy cardamom plants from all the locations surveyed and 30 isolates of *Trichoderma* sp. were isolated.



**Fig.6.** Symptoms of (a) rhizome rot and (b) root rot in cardamom

### Bacterial wilt

A new bacterial wilt disease on small cardamom was noticed in Wayanad, Kerala. Phenotypic and genetic characterization revealed that the causative organism is *R. solanacearum* biovar 3 phylotype 1. Multiplex-PCR based phylotyping, 16s rDNA and recN gene sequence based comparison and MLST based comparative genetic analysis further revealed that the strain is 100% similar to the ginger strain of *R. solanacearum*.

### Thrips - Source of resistance

Two hundred and forty one accessions of cardamom were screened for morphological characters such as plant type, nature (persistence) of bract and leaf sheath (firm or loose) and incidence of thrips (*Sciothrips cardamomi*) infested capsules. The mean percentage of infested capsules was 15.0, 23.4 and 32.5 in Malabar, Vazhukka and Mysore types, respectively. The mean percentage of infested capsules was 28.5 and 15.3 in accessions with persistent and non-persistent bracts, and 27.7 and 14.4 in accessions that had firm and loose leaf sheaths, respectively.

## TURMERIC

### CROP IMPROVEMENT

#### Genetic resources

One thousand and twenty six accessions of *Curcuma* spp are being maintained in the repository. Two hun-

dred and forty four accessions of turmeric received from NBPGR - Shillong and Thrissur were also planted for maintenance.

### Evaluation of seedling progenies

Out of 60 seedling progenies with higher multiplication rate during previous year, 25 lines showed higher multiplication rate (above 15 times) in the field. Rhizomes of 232 seedling progenies showed high variability for the quality parameters, curcumin, oil and oleoresin. Curcumin content ranged from 0.02 (354/3) to 4.92% (389/1) and 23 progenies showed above 3% curcumin. Forty nine progenies showed dry recovery of above 20%.

### CROP PRODUCTION

#### Organic farming

Among different systems of management organic and integrated systems recorded comparable yields (30.2 and 30.6 t/ha). The var. Alleppey Supreme has recorded higher yield (17%) under organic system than Prathiba. The soil organic carbon, N, P, K, Ca, Mg and Zn were higher under integrated system. The enzymes like acid phosphatases and dehydrogenase were also higher under integrated system. Among combination of organic manures, treatment with farm yard manure (FYM) + Neem cake (NC) + Vermicompost (VC) recorded highest yield (32 t/ha), followed by FYM+ Biodynamic formulation (BD501)+ Panchagavya (PG) (26.6 t/ha) compared to control (17.4 t/ha). The soil OC, N, P, Ca, Mg and Zn availability and acid and alkaline phosphatase activity were higher under FYM+NC+VC+PG and BD.

#### Micronutrients on yield and quality

The effect of Zn and B on the yield and quality of turmeric var. Prathiba was studied. Soil application of zinc @ 5 kg/ha and 0.25% Zn as two foliar sprays along with application of recommended dose of P recorded higher rhizome yield of 17.4 and 18.5 kg/3 m<sup>2</sup>, respectively. In the absence of P application, soil zinc application up to 10 kg/ha yielded higher (20.8 kg/3 m<sup>2</sup>) compared to other treatments. Similarly, without liming, application of B up to 1 kg/ha increased the rhizome yield (15.7 kg/3 m<sup>2</sup>) which was 39% higher compared to control. But when lime was

applied, similar increase in yield was observed even without application of B, indicating the benefits of correcting the soil pH in increasing the B availability. One foliar spray of B (0.2%) also recorded higher yield (16.7 kg /3 m<sup>2</sup>) on par with lime application alone. Curcumin content was significantly high (5.11%) in two foliar sprays of B @ 0.2%.

### Improved processing

Experiments on curing of turmeric (var. Prathiba) by improved steam boiling and conventional water boiling methods, showed that turmeric cured by traditional water boiling method for 40, 60, 90 min, took 11 days for drying while turmeric cured in improved boiler for 30, 45 and 60 min took 12, 23 and 24 days, respectively. Maximum retention of curcumin (5.91%) and essential oil (3.6%) was obtained in rhizomes cured by traditional boiling method for 40 min. Increase in curing time resulted in significant reduction in curcumin, starch, essential oil and oleoresin content. Slicing of rhizomes to 5 mm thickness and drying without curing, significantly reduced the drying time (9 days). However there was significant reduction in quality in terms of curcumin (5.71%) and essential oil (3.07%) contents.

## CROP PROTECTION

### Shoot borer - Bionomics

The life cycle of shoot borer was studied on four resistant and four susceptible accessions of turmeric. The average adult longevity was 3.8 and 4.0 days, on susceptible and resistant accessions, respectively. The fourth and fifth larval instar and pupal weights were 0.144, 0.114 and 0.087 g, respectively, on resistant accessions and 0.136, 0.123 and 0.085 g, respectively, on susceptible accessions. However, the differences were not statistically significant.

### Adoption of varieties

Turmeric variety Prathiba performed well in farmers' plots at Guntur, Andhra Pradesh and Gundlupet, Karnataka under different systems of planting and observed to be resistant to rhizome rot under field conditions and tolerant to water logging compared to the local types (Tekurpet and Salem). The average yield obtained at Guntur is 38.4 t/ha and the yield

estimate projection was 40 t/ha at Gundlupet.

### Impact Assessment

The survey was conducted in Erode and Bhavani taluks of Erode District in Tamil Nadu, a traditional turmeric tract and 25 farmers from each of the taluks were selected randomly to assess the level of adoption of scientific cultivation practices, mainly high yielding released varieties and its impact on yield and returns to the farmers. The survey revealed that, Suvarana (PCT-8), an improved variety released by IISR in 1987, has now spread over a period of 15 years, to an extent of about 80% of total area under the crop in Bhavani taluk followed by Roma, variety released from High Altitude Research Station, Pottangi to an extent of about 40 % in Erode taluk. The short duration of the crop (compared to local Erode and Salem varieties with around 10 months), suitable for early planting in places with assured irrigation, high curcumin content, relative disease tolerance compared local cultivars and stable yield over seasons are the reasons reported by the farmers for the adoption of the IISR variety, Suvarna. The farmers reported an yield of 35-40 t/ha for the improved varieties in Bhavani taluk for the irrigated crop. Due to high prices of turmeric prevailing in past two years, a B:C ratio of 11.84 was worked out for the region taking into consideration the price for the last five years.

## GINGER

## CROP IMPROVEMENT

### Genetic resources

Five hundred and ninety five accessions of ginger are being maintained besides 64 accessions received from NBPGR were also planted for maintenance. Among five shortlisted nematode tolerant accessions evaluated for morphological and yield characters, Acc. 219 was found to be promising with high yield and nematode tolerance.

### Cytology

A collection supplied by a farmer from Quilon, Kerala (as collection from China, Acc. 891) showed



vigorous plant morphology, variation in flower colour pattern, high pollen fertility and bold rhizomes. Pollen fertility assessed by staining was found to be 58% and *in vitro* germination was 50.58%. Pollen germination and pollen tube growth was observed on stigmatic surface and rarely in style also. However, no fruit set was observed. Cytological analysis of root tip cells showed that the collection is a tetraploid with  $2n=44$  (Fig 7).

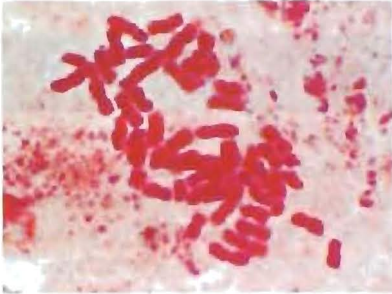


Fig 7. Tetraploid in Chinese ginger

## 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/5 m<sup>2</sup> yield targets in ginger were worked out and applied in 3-5 splits at Madikeri. Nutrient requirement for realizing all the three targeted yield levels could be predicted successfully with a positive deviation of 15.7 - 70.0%. The realized yield levels were 42.5, 47.8 and 52.0 kg/5 m<sup>2</sup> for the yield targets of 25, 35 and 45 kg/5 m<sup>2</sup>, respectively.

### Organic farming

Among the different management systems, highest yield was recorded under organic system (20 t/ha). In general var. Mahima and Varada performed well under organic management compared to Rejatha with 17-19% yield increase compared to inorganic system. The soil enzyme activity was higher under organic system. Among combination of organic manures, the treatment FYM+VC+NC+PG+BD has recorded highest yield (25.6 t/ha) compared to control (9.7 t/ha). The availability of major nutrients and acid and alkaline phosphatase and dehydrogenase

activity were higher under FYM+NC+VC+PG and BD application. Varada recorded higher oleoresin content under organic system (3.4%) whereas other varieties under integrated system.

## CROP PROTECTION

### Bacterial wilt - Characterization

Five new isolates of *Ralstonia solanacearum* biovar 3 from Calicut and Wayanad were added to the repository and were found to wilt ginger plants in 10-14 days. Multiplex-PCR based phylotyping of *R. solanacearum* revealed predominance of Phylotype I in India. An isolate from potato obtained from West Bengal tested positive for Phylotype II indicating its American origin. Multi-locus Sequence typing (MLST) of 21 strains of *R. solanacearum* was carried out by PCR amplification and sequencing of housekeeping genes (ppsA, adk, gapA, gdhA, gyrB) and virulence genes (hrpB, fliC and egl). Several novel alleles could be found in ginger strain of *R. solanacearum* on comparing with the alleles documented in the database [www.pamdb.org](http://www.pamdb.org). Real time PCR based detection of *R. solanacearum* in soil was standardized.

### Host resistance

Fifty four ginger mutants irradiated with gamma rays were challenge inoculated with *R. solanacearum* and two mutants showed resistant reaction even after three repeated inoculations.

### Disease management

Trials on management of bacterial wilt of ginger with 11 treatments, including two antagonistic plants (*Tagetes* sp. and *Ocimum sanctum*) and biopriming of rhizomes with six endophytic / rhizobacteria and an actinomycetes indicated that pre-planting with *Tagetes* sp. reduced the disease incidence and enhanced ginger yield. Nine actinomycete isolates from ginger soil were found to be antagonistic to *R. solanacearum*.

### Rhizome rot - Evaluation of biocontrol agents

Acetone + EtoAc extractions obtained from GRB 68 was promising in inhibiting *P. myriotylum* and *R. solanacearum* *in vitro*.

A pot experiment on the effects of PGPR, inorganic NPK fertilizers and their combinations on soil nutrient mobilization indicated that BRB 13 (*Enterobacter* sp.) and BRB 23 (*Micrococcus* sp.) applied in combination with 100% NPK (140-50-270 kg/ha NPK) registered higher levels of mineral N, Bray P and exchangeable K in soil. Besides, levels of microbial biomass-C, -N, -P, and hydrolytic enzyme activities were consistently higher in treatments with combined application of PGPR+NPK. Field experiments indicated that *Bacillus amyloliquefaciens* (GRB 35) and *Serratia marcescens* (GRB68) were promising for disease control and plant growth promotion. The strains GRB 68 (*S. marcescens*) and GRB 35 (*B. amyloliquefaciens*) were found to enhance the sprouting of rhizomes besides reducing the soft rot and bacterial wilt in ginger.

### Shoot borer - Bionomics

The life cycle of shoot borer (*Conogethes punctiferalis*) was studied on six resistant and six susceptible accessions of ginger. The mean adult longevity was 4.4 and 4.9 days, on resistant and susceptible accessions, respectively. The fourth and fifth larval instar and pupal weights were 0.104, 0.104 and 0.07g, respectively, in resistant and 0.127, 0.112 and 0.073 g, respectively, in susceptible accessions. However, the differences were not statistically significant.

### Evaluation of EPNs

The infectivity of EPNs strains IISR-EPN 01 to 08 was tested against shoot borer larvae under *in vitro* conditions. Among these, IISR-EPN 01, IISR-EPN 02, IISR-EPN 07 and IISR-EPN 08 were more pathogenic to the larvae causing 100% mortality within 72 h, whereas IISR-EPN 03, IISR-EPN 04 and IISR-EPN 06 killed all the larvae within 96 h.

## NUTMEG

### Genetic resources

The Plant Germplasm Registration Committee (NBPGR, New Delhi) approved the registration of the nutmeg germplasm A9-71 (IC-537220,

INGR10142) as a source of high sabinene (45.0% sabinene in nutmeg oil and 41.9% sabinene in mace oil). It had low levels of myristicin (1.9% and 1.1%), elemicin (0.8% and 1%) and safrole (0.1% and 3.2%).

### Budding

Green chip budding with orthotropic buds was successful in nutmeg on *Myristica fragrans* rootstock with 90-100% success. The ideal time for budding was August to November.

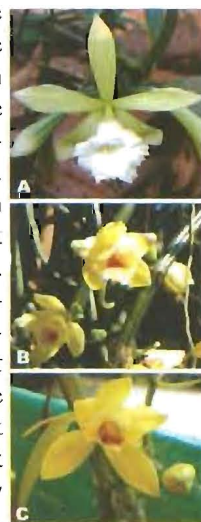
## CASSIA

Essential oil and oleoresin contents in scraped and unscraped dried bark from different portions of the shoot indicated that the oil content is high in scraped bark compared to the unscraped bark, whereas oleoresin content was high in unscraped bark.

## VANILLA

### Interspecific hybridization

Fifty interspecific hybrids each of *V. planifolia* x *V. tahitensis*, *V. tahitensis* x *V. planifolia* and selfed progenies of *V. tahitensis* were established *ex vitro*. One of the inter-specific hybrids between *Vanilla* sp. (A&N islands)-White flowered x *V. aphylla*, has flowered after 8 years of maintenance. The flowers were smaller than that of *Vanilla* sp (A&N), but larger than that of *V. aphylla*. There were 6-8 flowers per inflorescence. Flowers had the general appearance and colour of that of *V. aphylla*, but the frill of the labellum was more similar to that of *Vanilla* sp. (A&N). The plant was without any leaves in early years of growth but, later pro-



**Fig 8.** Flower types in interspecific cross in Vanilla: (a) *Vanilla* sp (A & N Islands), (b) *Vanilla aphylla*, (c) Interspecific hybrid



duced leaves smaller than that of *Vanilla* sp. (A&N). The results indicate a strong influence of *V. aphylla* which was used as male parent (Fig 8).

## ANTIOXIDANT PROPERTY OF SPICE EXTRACTS

The antioxidant property of extracts of *Garcinia indica*, *G. gummi-gutta*, tamarind and curry leaves were compared at different time periods, immediately after extraction, until one year after extraction, at three month intervals and quantified using *in vitro* methods, total antioxidant capacity by the phosphomolybdenum method, DPPH radical scavenging ability and Fe(III) to Fe(II) reducing activity and quantifying the total phenols. Drastic decrease in the antioxidant parameters has been reported after six months of extraction. Chemoprofiling of curry leaf essential oil revealed that, *l*-caryophyllene which was up to 26% after six months of extraction was reduced to negligible amounts (0.5%) by nine months due to oxidation to *l*-caryophyllene oxide.

A comparison of the essential oil of mature and tender curry leaves revealed 33% and 35% of *l*-caryophyllene and 11% and 9% of  $\beta$ -phellandrene and  $\alpha$ -selinene in mature and tender leaves, respectively. The essential oil yield and total phenol content of tender and mature curry leaves were at par. The DPPH radical scavenging ability of essential oil and water extract of tender leaf was significantly higher than the mature leaves (78% and 52% respectively), while the Fe(III) to Fe(II) reducing activity of essential oil and ethanol extract of tender leaf were significantly higher than that of mature leaves (83% and 45% respectively).

## BIOINFORMATICS

Secretome analysis of *P. capsici* and *R. similis* was carried out. On exploring the ESTs of *R. similis*, 214 secretory proteins were identified and their functional annotation was carried out. About 45% of these secretory proteins showed similarity to nematodes. The mode of interaction of Glucanase Inhibitor Protein (GIP) from *P. capsici* with plant endo-  $\beta$ -1, 3-glucanases was stud-

ied through structural and docking studies.

## Database development

New databases on ginger and turmeric germplasm accessions were developed and hosted on the Institute server. The scope of Phytolib, the literature database, was further widened to include articles on *Ralstonia* and *Fusarium*. PhytoPD, a repository of Polymerase Chain Reaction primer sets, useful for the identification and detection of *Phytophthora* species, was developed and uploaded. It includes all the universal primers and species-specific primers for more than 30 species of *Phytophthora* published in literature.

## National consultative meet

Hortinformatics 2010, the National consultative meet on Bioinformatics in Horticulture was organized 11-12 October 2010. About 90 delegates from more than 30 research institutes and universities participated in the event. Sixteen invited talks and 23 posters were presented by various scientists in four technical sessions during the meet (Fig 9).



Fig 9. DDG (Hort) inaugurating Hortinformatics 2010

## EXTENSION

The extension and training services of the institute is coordinated through the ATIC, a single window system of delivery of technology services, inputs and products to the end users. During the year, 2769 farmers availed farm advisory services from the centre; 1175 students visited the centre for study purpose. An income of ₹ 246,725 was generated through the sale of planting material, bioagents, spice produce and publications.



The institute conducted three on-campus training programmes sponsored by CPCRI, Kasaragod, Department of Horticulture, Maharashtra and Department of Agriculture, Punjab, in which 35 scientists, officials and progressive farmers participated. One off-campus training was organized under the Horticulture Mission for North East and Himalayan States at Guwahati, Assam in which 23 SMSs from various KVKs and 27 progressive farmers participated. The institute participated in three exhibitions/farmers fairs at the national level and three at the regional/state level. Two video conferencing sessions with village resource centers in Wayanad district were organized through VSAT facility in which 105 farmers participated.

**Mobilising mass media support for sharing agro-information**

- Media visits were organized to farmers plots in Gundlupet (turmeric), Appangala (black pepper) and Kayamkulam (coconut)
- More than 30 success stories and 75 news items have been given through print media (on coverage of media meet, Kisan mela, success stories, media visits, technologies released etc.)
- Four radio news based programmes and 6 TV news clippings and four episodes of programmes on medicinal values of ginger, turmeric, black pepper and cardamom for regional TV channel were provided
- Fifteen audio capsules were produced and broadcasted through AIR Calicut
- Three video films on Augmenting Black Pepper production - A success story (Malayalam, English, Hindi), Success story of a 'Prathiba' grower - Post production stage and Success of broiler goat technology - Post production stage were produced

**KRISHI VIGYAN KENDRA**

**Trainings**

Nine on-farm trials and eight front line demonstrations in horticulture, fisheries, poultry, livestock and food technology are in progress. One hundred and sixty six training programmes were conducted during the year in various subjects in which 4026 farmers, unemployed youth and women participated. Participatory seed production was undertaken in two ginger and three tur-

meric farmers' plots and the seed rhizomes obtained were sold to 42 and 69 farmers, respectively. The KVK also participated in five exhibitions and six study tours were arranged for farmers.

**Karshika Sankethika Darshanam**

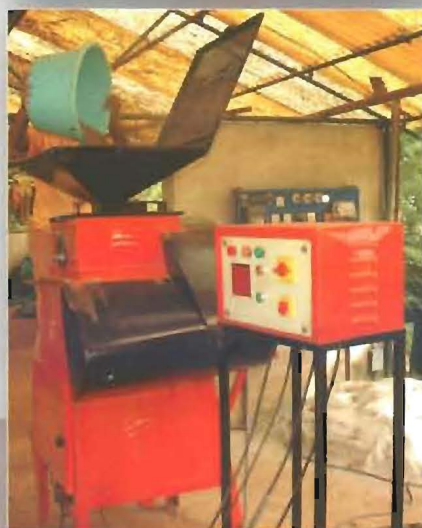
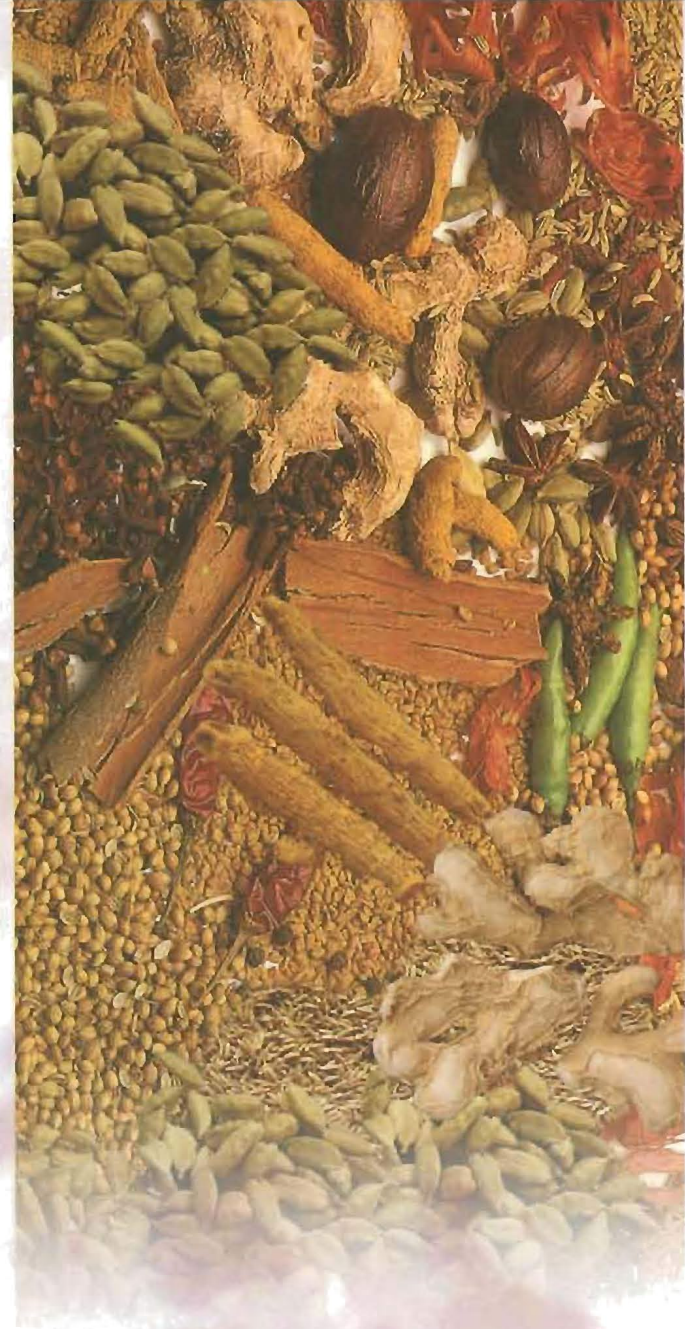
Karshika Sankethika Darshanam 2011, Kisan Mela & Technology Expo was organized from January 27-29, 2011 at IISR, Chelavoor Campus. Mr. K K Raghavan, Joint Rubber Production Commissioner, Rubber Board, Calicut, inaugurated the mela. Dr. V A Parthasarathy, Director of IISR chaired the inaugural session. Dr. I John Kutty, Associate Director of Research, KAU, Corporation Councillor Mr. M P Hameed and Dr. C V Sairam, Principal Scientist, Agri Economics, Zonal Director Unit, KVK Bangalore offered felicitations. Fifteen exhibition stalls of different organizations displayed their technologies/ products. Over 200 farmers and 500 students attended the three days programme. A quiz programme on Agriculture for school students was also organized. Dr. T Pradeep Kumar of KAU, Dr. Manju Sasidharan of KVVU and Mr. R Suresh, Project Officer, HortiCorp delivered specific talks to farmers.



**Fig.10** View of exhibition organized for Kisan Mela

**HUMAN RESOURCE DEVELOPMENT**

- One month summer training on Biochemistry, Biotechnology and Bioinformatics was conducted for 19 M.Sc. students during 5th May - 4th June 2010.
- Thirteen M.Sc. and one M. Phil students carried out project work in various disciplines. Three students were awarded and two have submitted for Ph. D degree.



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