



# Annual Report

## 2003-04



IISRAR-16

Indian Institute of Spices Research  
Calicut



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

**Correct citation**

Indian Institute of Spices Research, Annual Report 2003-04, Calicut.

**Publisher**

*Director*

Indian Institute of Spices Research  
Calicut – 673 012, Kerala, India.

**Editor**

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**Hindi translation**

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**Front cover**

Varieties of black pepper and turmeric proposed for release

**Cover design**

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ISBN 81-86872-20-5

September 2004

**Printed at**

Modern Graphics, Kochi

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# Preface

The activities of Indian Institute of Spices Research for the year 2003–04 received an impetus with the approval of the EFC to the X<sup>th</sup> Plan proposal of the institute to a tune of Rs. 735 lakhs. The research programmes of this institute were grouped under 11 mega projects with a view to orienting the research programmes on problem fronts rather than on disciplines. The genetic resources were enriched with further collections having been added to the gene banks of black pepper, cardamom, ginger, turmeric and vanilla. These germplasm were also being characterized using IPGRI descriptor. As envisaged in the X<sup>th</sup> Plan the monitorable targets for the Plan are breeding of pepper tolerant to *Phytophthora* foot rot, development of control measures for soft rot of ginger and isolation and characterization of bioactive produces of turmeric and ginger. In this direction, a variety IISR-Shakthi showing tolerance to *Phytophthora* foot rot was recommended for release in the XVII National Group Meeting of Research Workers of All India Coordinated Research Project on Spices held during 3–5 February 2004. In addition, 3 black pepper varieties namely, IISR Thevam, IISR Girimunda and IISR Malabar Excel and two varieties of turmeric namely, IISR Alleppey Supreme and IISR Kedaram were proposed for release. Research programmes on drought management was also intensified in black pepper and cardamom. Application of molecular tools for characterizing wilt pathogen affecting ginger

led to the standardization of ARDRA techniques. The emerging menace of root mealy bug of pepper is being tackled through an ICAR Cess Fund Project sanctioned during the year. More than 1500 farmers took the help of IISR for advice while the KVK organized 76 training programmes for farmers and extension functionaries. The Bioinformatics Centre organized a two day workshop on agriculture bioinformatics and 21 days training programme on bioinformatics. During the year IISR hosted the XVII National Group Meeting of Research Workers of All India Coordinated Research Project on Spices during 3–5 February, 2004. The achievements are only tip of the ice berg and we have miles to go before the entire problem of spice growers are answered.

I deem it a great privilege to place on record my grateful thanks to Dr. Mangala Rai, Director General for giving all support to our institute and also making me as a member of the Governing Body. The encouragement, support and guidance received from our knowledgeable Deputy Director General, Dr. G. Kalloo, needs special mention. Without his support we would not have achieved an iota of what has been reported. I am highly grateful to him. I also thank Dr. S. N. Pandey, Asst. Director General (Horticulture and P.C.) and Dr. K. V. Ramana, Project Coordinator (Spices), for the support rendered to me. I compliment Dr. J. Rema, Senior Scientist, for editing this Annual Report.





# प्रस्तावना

भारतीय मसाला फसल अनुसंधान संस्थान का वर्ष 2003-04 की कार्यविधियाँ संस्थान के दसवीं योजना प्रस्ताव के इ एफ सी के अनुमोदन के द्वारा 735 लाख रुपये मिलने से बढ़ा दी। अनुशासन की अपेक्षा समस्या प्रधान अनुसंधान कार्यक्रमों को प्रमुखता देकर संस्थान के शोध कार्यों को 11 महा योजनाओं के अन्तर्गत बाँट दिया। काली मिर्च, इलायची, अदरक, हल्दी और वैनिला के जीन बैंक में और भी कलक्शनों को जोड़कर जननिक संसाधनों की वृद्धि कर दी। जर्मप्लासम को आई पी जी आर आई डिस्क्रिप्टर द्वारा चरित्रांकित किया जा रहा है। X वीं योजना में प्रस्तुत प्लान के लिए प्रबोधित लक्ष्य (i) फाइटोफथोरा खुर गलन के सह्य काली मिर्च प्रजनन, (ii) अदरक के मृदु गलन के लिए नियन्त्रण उपायों का विकास (iii) हल्दी और अदरक के जैव सक्रिय उपजों का पृथक्करण और चरित्रांकन आदि है। इस निर्देश के आधार पर फाइटोफथोरा खुर गलन के सह्य प्रजाति पी 24 को 3-5 फरवरी 2004 को आयोजित अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना के शोध कर्मियों के राष्ट्रीय दल बैठक (17वीं कार्यशाला) में निकालने की सिफारिश की। इसके अलावा काली मिर्च की तीन प्रजातियाँ - आई आई एस आर तेवम, आई आई एस आर गिरिमुंडा और आई आई एस आर मलवार एक्सल तथा हल्दी की दो प्रजातियाँ - आलप्पी सुप्रीम और केदारम को विमोचित करने का प्रस्ताव किया। काली मिर्च और इलायची के सूखापन प्रबन्धन के लिए शोध कार्यक्रम तीव्र बना दिया। म्लानी (विल्ट) रोगबाधित अदरक का चरित्रांकन करने के लिए आणविक उपकरणों (मोलिकयूलर टूल्स) का प्रयोग करने से ARDRA प्रविधियों का मानकीकरण हो जाता है।

प्रस्तुत साल मंजूर किये ए पी सेस फन्ड प्रोजेक्ट द्वारा काली मिर्च के मूल में आक्रमण करनेवाले मीली बग को नष्ट कर दिया जा सकता है। आई आई एस आर से सहायता पाने के लिए 1500 से अधिक किसान परामर्श ले रहे जबकि कृषि विज्ञान केन्द्र ने किसानों और विस्तार कर्मियों के लिए 76 प्रशिक्षण कार्यक्रम आयोजित किये। जैव सूचना केन्द्र ने कृषि जैव सूचनाओं पर दो दिन की कार्यशाला एवं जैव सूचनाओं पर 21 दिन का प्रशिक्षण कार्यक्रम आयोजित किये। प्रस्तुत साल 3-5 फरवरी 2004 को अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना के शोध कर्मियों का राष्ट्रीय ग्रूप मीटिंग (17 वीं कार्यशाला) आई आई एस आर में चलाया गया। मसाले उत्पादकों की सारी समस्याओं को हल करने के लिए हमारी देन बहुत कम होती है।

इस संदर्भ में डॉ मंगला राय, महा निदेशक के प्रति, जिसने अपने संस्थान की कई मदद की है और मुझे शासी निकाय का सदस्य भी नियुक्त किया है, अपनी कृतज्ञता प्रस्तुत करना मेरा विशेष अधिकार मानता हूँ। हमारे जाने माने उप महा निदेशक, डॉ जी कल्लू, जिसने हमें प्रोत्साहन, मदद और मार्गदर्शन दिया है जिसका नाम विशेष रूप से प्रस्तुत करने की आवश्यकता है। जो कुछ हम पा गये हैं सब उनकी मदद से है। मैं उनसे अत्यन्त आभार प्रकट करता हूँ। मैं डॉ एस एन पाँडे, सहायक महा निदेशक (हॉर्टी & पी सी) और डॉ के वी रमणा, परियोजना समन्वयक (मसाला) को भी उनके द्वारा प्रस्तुत सहायता के लिए धन्यवाद ज्ञापित करता हूँ। इस वार्षिक रिपोर्ट के संपादन के लिए मैं डॉ जे रमा, वरिष्ठ वैज्ञानिक का सम्मान करता हूँ।



# Executive Summary

The Indian Institute of Spices Research, Calicut, has the mandate for conducting research in spices and has been serving as a nodal research institute for spices for over a quarter century. The research highlights and other major activities of the institute are summarized here.

## Genetic resources

Surveys were conducted and 51 accessions of black pepper, 3 accessions of *Amomum subulatum*, 1 accession of *Alpinia* sp., 11 accessions of *Garcinia indica*, 1 accession of *G. gummi-gutta*, 30 accessions of *Vanilla planifolia*, including a collection having multi-branched inflorescence, and 7 accessions of paprika were collected and added to the germplasm during the year. The *in vitro* genebank has been strengthened with the addition of 10 accessions each of black pepper and cardamom, 36 accessions of ginger and 27 accessions of turmeric making the total to 750 accessions.

One hundred accessions of black pepper were characterized based on the IPGRI descriptor. Inter-Simple Sequence Repeat (ISSR) was successfully used in diversity analysis of *Piper*. Forty nine accessions of cardamom were also characterized and catalogued based on IPGRI descriptor. Incidence of leaf blight and rhizome rot was recorded in 114 accessions of cardamom and 1 highly resistant, 14 resistant, 54 tolerant, 35 susceptible and 10 highly susceptible accessions were identified. Over 100 selected accessions of cardamom germplasm were characterized using RAPD, ISSR and PCR-RFLP profiles. RAPD profiling of 96 accessions of turmeric using 15 random decamer primers and 96 ginger accessions

using ten random decamer primers indicated good polymorphism among the turmeric accessions and less polymorphism in ginger.

The black pepper accession Coll. 1041 (IC-316598) (for its field tolerance to foot rot disease) and turmeric accession 657 (IC-296550) (for high yield and high curcumin content) were registered with National Bureau of Plant Genetic Resources, New Delhi.

## Crop improvement

Four black pepper lines namely, OPKm (early maturity, high yield, bold berries, long spike and tolerance to drought), HP-728 (early maturity and high yield), HP-780 (high yield and high driage) and HP-1411 (high yield) are in the advanced stage of evaluation and continued to perform well in farmers' fields.

Crossing was carried out in 19 combinations of cardamom using 6 parents selected for specific characters (high yield, high quality, tolerance to drought, tolerance to rhizome rot and resistance to leaf blight and mosaic disease) and the fruit set varied from 22%–66%.

The elite lines A9-20, A9-5 and A9-53 performed better than other lines in the progeny evaluation trial of nutmeg at Peruvannamuzhi. In the progeny evaluation trial of cassia at Appangala, the performance of the elite line B-3 was superior to other entries.

Among the various species of *Myristica* evaluated as rootstocks for grafting nutmeg to increase productivity, performance of *M. malabarica* was superior to others in the third year of planting. A clonal material of *Syzygium heynianum* (a rootstock compatible

with clove) was multiplied and grafted with clove as scion for further evaluation.

Pruning of *V. andamanica* accessions during October resulted in profuse flowering in one accession and induced flowering for the first time in four others. Intervarietal crosses and selfing were done in white and purple flowered accessions of *V. andamanica* and fruit set was achieved. Successful interspecific crosses between *V. andamanica* and *V. aphylla* were made.

Five plants regenerated from *agrobacterium* treated leaf explants of black pepper in selection medium containing kanamycin were planted out. Ten more plants were regenerated using hypocotyls explants. Studies are in progress to ascertain the stable expression of osmotin the gene of interest in the putative transgenics. Gene specific primers were utilized for amplification of putative chitinase and *Phytophthora* resistance genes. The 3' end of the corresponding cDNAs were sequenced.

Cryopreservation of *P. barberi*, an endangered species and *V. planifolia* using encapsulation-vitrification method was standardized. A protocol for isolation of DNA from powdered market samples of turmeric has been standardized.

### High yielding varieties

Four black pepper varieties namely, IISR-Thevam (a selection of Thevamundi with field tolerance to foot rot disease coupled with high yield), IISR-Girimunda (a hybrid suited to high altitude areas) and IISR-Malabar Excel (a hybrid rich in oleoresin) IISR-Shakthi (OP progeny of Perambaramunda, tolerant to foot rot disease with high yield and high driage) and two turmeric varieties namely, IISR-Alleppey Supreme (a clonal selection of Alleppey Finger Turmeric) and IISR-Kedaram (a germplasm selection, rich in curcumin and tolerance to leaf blotch) were proposed for release.

### Evaluation for quality

Among the 77 cardamom germplasm

accessions evaluated, APG-246, 248, 352 and 378 had more than 8% oil. APG 357 and 365 contained 42%  $\alpha$ -terpinyl acetate with relatively low cineole.

Among the 400 turmeric accessions evaluated, Accs. 103, 114, 547 and 575 had more than 5% volatile oil. Accs. 103 and 547 contained 16.5% oleoresin; and Accs. 240, 592 and 593 had 5.5% curcumin. The *phenylalanine ammonia lyase* was found to be the rate-limiting enzyme in curcumin biosynthesis in turmeric. The enzyme was also found to be associated with the microsomal fraction. Based on the incorporation studies using  $^{14}\text{C}$ -phenylalanine, the initial precursor in the biosynthetic pathway was confirmed as phenylalanine.

Bark of *Cinnamomum burmannii* (Padang cassia) contained 2.8% oil and 11.8% oleoresin and *C. loureirii* (Vietnam cassia) contained 3.3% oil. The colour value of paprika accessions ranged from 39 to 278 ASTA units with a mean value of 184 ASTA units and coefficient of variation 2.46%.

### Mixed cropping system

Intercropping *P. chaba* (with about 4000 plants on *Glyricidia* standard per hectare) in arecanut plantation provided an additional income of Rs. 32,400 ha<sup>-1</sup>. In addition, the support tree would enrich the soil by fixing atmospheric nitrogen and would provide green leaf for mulching.

### Integrated plant nutrient management

Application of zinc significantly increased the soil zinc availability in ginger and the highest concentration was obtained with an application of 15 kg Zn ha<sup>-1</sup>. A cubic model satisfactorily explained the relationship between mean rhizome yield and the fertilizer application rate. A fertilizer dose of 6 kg ha<sup>-1</sup> of Zn was optimized from the model for getting maximum rhizome yield and the maximum limit of soil DTPA-Zn for obtaining high rhizome yield was 3.4 mg kg<sup>-1</sup>.

## Drought management

Forty five cardamom genotypes were screened for relative water content, specific leaf weight and stomatal count. They recorded significant variations for all the characters studied. Three collections with high biomass and yield were collected from farmers' fields in Kodagu District for further evaluation for drought tolerance. Introducing contour staggered trenches in coffee and cardamom plantations was effective in conserving soil and water.

## Disease management

### *Phytophthora foot rot of black pepper*

Seedling progenies of black pepper were raised both by selfing popular varieties (Panniyur-1, Subhakara and *Phytophthora* tolerant P-24) and by hybridizing two agronomically superior varieties (Panniyur-1 and Subhakara). Among the selfed progenies, the progenies of P-24, Subhakara and Panniyur-1 showed 13.85%, 9.7% and 7.2% resistant reaction respectively. Out of 169 hybrid progenies of black pepper involving the two susceptible parents, Panniyur-1 and Subhakara, 7.15% showed resistant reaction.

The performance of black pepper grafts on *P. colubrinum* (resistant to *Phytophthora capsici*) was satisfactory in marshy areas. It was recommended to grow five grafts around each support tree (arecanut) to get maximum coverage and yield.

Promising isolates of rhizobacteria suppressing both *Radopholus similis* and *Meloidogyne incognita* were screened against *P. capsici*. The isolates IISR-658, IISR-853 and IISR-869 inhibited *P. capsici* in laboratory tests. These three isolates were also efficient phosphorous solubilizers.

### *Stunt disease of black pepper*

Black pepper plantations of Dakshina Kannada, Madikeri, Udupi, and Uttara Kannada districts of Karnataka and Idukki, Kannur, Kasaragod, Kozhikode and Wyanad

districts of Kerala were surveyed for the incidence of stunt disease. The disease distribution and incidence were higher in Kerala compared to Karnataka. High incidence of the disease was noticed in black pepper plantations situated at higher altitudes in Idukki and Wyanad districts.

Serological analysis of the stunt disease affected black pepper indicated the involvement of two viruses namely, cucumber mosaic virus (CMV) and a badnavirus. The mealy bug *Ferrisia virgata* was identified as the vector transmitting the badnavirus.

### *Spike shedding in black pepper*

Observations recorded in various crop combinations, input management and light profiles indicated that the spike shedding varied from 9% to 87% in different field situations. Highest spike shedding was noticed in the rainfed blocks and heavily shaded conditions.

Anthracnose disease (caused by *Colletotrichum gloeosporioides*), predominance of female flowers, lack of pollination in rainfed areas, heavy shade and delayed emergence of spike were identified as the major reasons for spike shedding. The spike infection in Panniyur-1 varied from 13.0% to 83.4% and highest infection was recorded in the plants growing under heavy shade.

The varieties Panniyur-5, Panchami and Subhakara showed field tolerance to anthracnose infection and Panniyur-1 and Panniyur-3 were highly susceptible. Among 14 cultivars Chomala, Thevanmundi, Karimunda, Chettalli selection, Aimpirian and Arakalamunda showed field tolerance to anthracnose.

Irrigation of black pepper vines 4-5 times at an interval of 5-7 days @ 40-50 litre plant<sup>-1</sup> commencing from 22 March, followed by shade regulation of support trees to provide minimum 7,500-10,000 lux light under cloudy condition is optimum for managing spike shedding in high altitudes. The irrigation

coupled with recommended phytosanitary, prophylactic and nutrition management practices are necessary for holistic management of spike shedding and anthracnose in high altitudes.

#### **Bacterial wilt of ginger**

DAS-ELISA was standardized for detection of *Ralstonia solanacearum* in soil and ginger rhizome. *R. solanacearum* could not be detected from soil and periderm of ginger using this technique, indicating that the pathogen do not survive in the debris of bacterial wilt affected ginger plants, which was further confirmed by bioassay in green house.

Amplified Ribosomal DNA Restriction Analysis (ARDRA) technique was standardized for molecular characterization of *R. solanacearum*. The biovars of *Ralstonia* could be differentiated using ARDRA with Sau 1A and MSP 1 restriction enzymes. Molecular analysis of 33 isolates of *R. solanacearum* using Rep-PCR and ITS PCR revealed existence of single virulent lineage of *Ralstonia* causing bacterial wilt of ginger.

Antibacterial activities of cell free culture filtrate of *T. viride* was found effective against *R. solanacearum*. The culture filtrate was active against the growth and multiplication of the pathogen *in vitro*. The culture filtrate was inactivated by heat at 70–90 °C. Mutant derivative (albino) of *T. viride* did not produce antibacterial metabolites.

Effectiveness of rhizome solarization in disinfecting *R. solanacearum* was proved using post enrichment DAS-ELISA with *R. solanacearum* specific antibodies. Field trials on bacterial wilt management using seed disinfection through rhizome solarization revealed that the strategy could be effectively used for managing the disease.

Endophytic bacteria associated with ginger rhizome and pseudostem were isolated from bacterial wilt affected ginger plants as well as from healthy plants.

#### **Soft rot of ginger**

Two hundred accessions of ginger were screened for their reaction to soft rot disease of ginger caused by *Pythium sp.* Accessions 261, 269 and 274 were moderately tolerant to the disease.

Studies with different isolates of plant growth promoting rhizobacteria (PGPR), against soft rot disease of ginger showed that PGPR isolates IISR-13, IISR-51, IISR-151, IISR-152 and IISR-906 were effective in reducing the disease incidence to less than 5%. Seed treatment with metalaxyl (Ridomil MZ-72 wp) at 0.125% and soil application of the same twice along with neem cake reduced the disease incidence to less than 10%.

#### **Diseases of vanilla**

Disease survey was carried out in 22 vanilla plantations distributed in Calicut, Idukki and Wyanad districts of Kerala and 14 plantations in Dakshina Kannada, Udupi and Uttara Kannada districts of Karnataka. Two viral diseases namely, mosaic and necrosis were observed in most of the plantations surveyed and the incidence ranged from 0 to 10%. A disease caused by *C. gloeosporioides* and characterized by premature yellowing and bean shedding was noticed in almost all the vanilla plantations of lower elevations (<100 m). High temperature (>30 °C) and low relative humidity (<60%) were identified as the predisposing factors for the occurrence of the disease.

#### **Nematode management**

Fifty each of ginger and turmeric germplasm accessions were screened against *M. incognita*. Out of these, 14 ginger and 13 turmeric accessions were short-listed for the second round of screening. Nematode resistant accessions namely, Acc. 202 in ginger and Acc. 82 and Acc. 84 in turmeric were found superior in yield and quality characters. An *in situ* screening experiment was laid out at Peruvannamuzhi farm to confirm the resistance of nine black pepper germplasm accessions.

One hundred and three endophytic bacteria from black pepper plants were isolated through different approaches. These isolates were characterized by colony morphology, antibiotic sensitivity, their ability to colonize black pepper plants and nematicidal properties. The nematicidal activity of these isolates varied from 0–29%. *In silico* analysis for developing primers specific to biocontrol genes has been completed. A novel method to differentiate live and dead nematodes was developed. Genomic DNA of nematicidal rhizobacteria isolated from black pepper, cardamom, ginger etc. was isolated through the CTAB-SDS method and procedure for ARDRA finger printing was standardized.

The PGPR, IISR-522, IISR-528 and IISR-658 suppressed plant parasitic nematodes and significantly reduced yellowing in black pepper. Application of IISR-13, IISR-51 and IISR-866 in ginger field increased the yield by 31.8% to 56.4%. Among the four fungal bioagents evaluated, *Verticillium chlamydosporium* and *Scopulariopsis* sp. were effective to manage the nematode infestation in black pepper.

### Insect pest management

#### *Pollu beetle of black pepper*

One hundred and sixty five accessions of cultivars and 42 accessions of hybrids of black pepper available in the Germplasm Conservatory were screened against *pollu* beetle (*Longitarsus nigripennis*).

#### *Root mealybug of black pepper*

Surveys were conducted in 162 black pepper gardens at 54 locations in Kasaragod, Kannur, Kozhikode and Idukki districts in Kerala and in 50 gardens at 18 locations in Dakshina Kannada, Udupi, Uttara Kannada and Hassan districts in Karnataka to study the distribution of root mealybug (*Planococcus* sp.) on black pepper. Six gardens in five locations in Idukki District and five gardens

in two locations in Karnataka were infested by the pest.

#### *Shoot borer of ginger*

Five hundred and fifty one accessions of ginger available in the Germplasm Conservatory were screened against shoot borer (*Conogethes punctiferalis*) and none of the accessions had less than 5% shoots infested by the pest; 69 accessions had 5-10% shoots infested by the pest.

Neem oil and a commercial neem product (1% concentration each) that were promising against shoot borer during the previous year when sprayed at fortnightly intervals during July to October were not effective this year in reducing the pest infestation on ginger.

Dried rhizomes of 77 accessions of ginger and 12 accessions of turmeric were screened for damage by cigarette beetle (*Lasioderma serricorne*) and rhizomes of 2 accessions of ginger were free of pest infestation.

### Economics

Surveys conducted on cultivation of vanilla in Kerala, Tamil Nadu and Karnataka indicated that there is a rapid increase in vanilla cultivation due to the prevailing better prices for the crop. The estimated cost of cultivation indicated a benefit cost ratio of 3.3 and 3.8 in Karnataka and Kerala respectively. The technologies developed under the NATP projects for sustainable cultivation of cardamom and black pepper in Coorg District of Karnataka were evaluated for its economic viability.

### Training and development programmes

The institute organized two training programmes on production technology of spices and one programme each on on-farm processing of spices and viral disease management and nursery management in black pepper for field extension functionaries of State Department of Agriculture and Horticulture and research workers of ICAR institutes and agricultural universi-

ties. Training programmes on "Biotechnology/Biochemistry and Bioinformatics" for M. Sc. students, "Experimental Designs and Computer Applications in Spices Research" for the scientist of All India Co-ordinated Research Project on Spices and "Biotechnology of Black pepper" for a scientist from Sri Lanka and 'Cultivation and Processing of Spices' for a delegate from Eritrea were organized. Scientific, administrative and technical staffs of the institute were deputed to various institutes within the country for training in their respective fields of specialization. Forty short-term research projects were carried out by the post graduate students from various universities under the guidance of the scientists of the institute. The Consultancy Processing Cell offered training programmes, consultancy services and transferred technologies developed by the institute and earned an income of Rs. 6,86,000.

#### **Production of planting material**

Five lakh rooted laterals of black pepper, 25,799 cardamom seedlings, 1,226 cardamom suckers, 2 tonnes each of ginger and turmeric seed rhizomes, 649 garcinia grafts and 6,000 nutmeg grafts were produced and distributed to farmers and other developmental agencies.

#### **Agricultural Technology Information Centre**

Planting materials of improved varieties of spices and *Trichoderma* culture were distributed to farmers and other agencies through the single window delivery system of Agricultural Technology Information Centre (ATIC). 1,572 farmers were offered advisory services on various aspects of spice cultivation and 529 students from various schools visited the centre. ATIC generated an income of Rs. 1,48,786 through sales of planting material, *Trichoderma* culture, extension pamphlets and testing of soil and manure.

#### **Krishi Vigyan Kendra**

Krishi Vigyan Kendra (KVK) conducted 91 training programmes for farmers, unemployed youth, school drop outs and extension functionaries in which 1754 trainees, including 765 women participated. An advanced training programme of 1 month duration was organized for 20 educated, unemployed rural women in bamboo handicrafts to equip them for self employment. The KVK conducted frontline demonstrations in farmers' fields on high yielding okra (var. Arka Anamika), high yielding cowpea (var. Arka Garima and Sharika) and Kuttanad layer ducks and the results were encouraging. On-farm testing trials were conducted in fruit fly management in bittergourd, utilization of grafted pepper in swampy areas and testing the effectiveness of prostaglandin  $F_2\alpha$  in anoestrus dairy cattle.

The KVK organized 2 *kisan melas* cum exhibitions, delivered radio talks and published popular articles for disseminating the agricultural technologies developed at the institute. Five study tours were organized for farmers to visit various research institutions and farms. The animal clinic took up 475 consultancy/advisory/home services and 311 artificial inseminations. Several rural youth started self employment avocations in agri-nurseries, vermicomposting, fruit processing, production of handicrafts and goatary with the help of the KVK. The KVK generated an income of Rs. 4,06,832/- through sale of planting materials of spices, fruits, plantation crops, ornamentals etc., and Rs. 22,395 through the animal clinic. The KVK was awarded a Commendation Certificate by National Bank for Agricultural and Rural Development for its outstanding achievements in the field of Vikas Volunteer Vahine clubs.

#### **Bioinformatics Centre**

Bioinformatics centre organized a two-day workshop on "Agricultural Bioinformatics"



and a 21 days training programme on "Bioinformatics and Biotechnology – Tools and Applications". The centre also collaborated with the Department of Electronic Accreditation of Computer Courses, Calicut in conducting courses in bioinformatics.

Four databases namely, *Phytophthora* information resource: a comprehensive web resource for the *Phytophthora* species; *Curcuma* species; *Myristica* species and nutmeg germplasm; chemical constituents of cardamom oil and their metabolic pathways and two softwares namely, Phytfinder (for identification of *Phytophthora* species based on their morphological characters) and Biovarchar (for biovar characterization of *R. solanacearum*) were developed.

#### **Integrated National Agricultural Resources Information System (INARIS)**

An interactive expert system for vanilla was developed and the expert system for black pepper (pepper anthology) was updated. A digital code book for spice crops was developed and the spice database was strengthened. Using the geo-database on spices a spice atlas was developed to focus on the district wise distribution of spice crops.

#### **All India Co-ordinated Research Project on Spices**

The All India Co-ordinated Research Project on Spices (AICRPS) is the largest network in the country to conduct and co-ordinate the spices research in 19 co-ordinating and 8 voluntary centres.

#### **Crop improvement**

The AICRPS centres strengthened the genetic resources of spice crops and at present, the germplasm holdings consist of 650 accessions of black pepper, 369 accessions of cardamom, 644 accessions of ginger, 1307 accessions of turmeric, 228 accessions of tree spices and 3901 accessions of seed spices. The germplasm was evaluated for various parameters and the promising accessions in each crop were identified.

At Ambalavayal, the black pepper varieties, Panchami, Panniyur-2, Panniyur-3, Panniyur-4 and accessions 2426 and 2445 were found promising for the high range regions of Kerala. The cardamom accessions, CL-692, CL-730 and D-237 were identified as promising at Mudigere and has been included in varietal evaluation trials.

In ginger, highest fresh rhizome yield was recorded in V<sub>3</sub>S<sub>1</sub>-8 (28.25 t ha<sup>-1</sup>) at Pottangi. The dry matter content of ginger accessions varied from 13.0% to 22.5%. The oleoresin and essential oil content ranged between 4.00% to 9.67% and 0.25% to 2.00%, respectively, and crude fibre varied from 3.93% to 5.95%. In turmeric, highest fresh rhizome yield was recorded in PS-39 (26.62 t ha<sup>-1</sup>) under initial evaluation trial at Pottangi. At Solan, five collections namely, ST-365, BDJR-1244, Cls-29, PTSS-24 and DKH-26 recorded an increase in yield to the extent of 17.64 – 32.62% over the check.

For successful hybridization in cumin, emasculation was recommended to be carried out before 10 am in slightly pink and unopened flower bud and pollination on the next day or third day or second and third day (twice) after emasculation between 11 am to 7 pm. Fenugreek accessions, HM-444 (a green seed coat mutant) and HM-372 and HM-376, (yellow seed coat mutants) gave highest seed yield of 23.9 q ha<sup>-1</sup>, 33.85 q ha<sup>-1</sup> and 32.65 q ha<sup>-1</sup>, respectively. HM-444 is also resistant to both downy mildew and powdery mildew diseases. At Jobner, RTP-4 recorded highest yield (1518 kg ha<sup>-1</sup>) followed by RTP-8 (1477 kg ha<sup>-1</sup>) and RTP-9 (1471 kg ha<sup>-1</sup>), and would be evaluated under CVT. Several varieties/lines were identified for yield and quality attributes through CVT/CYT.

#### **Crop management**

In black pepper-arecanut mixed cropping system, application of 20 litres water vine<sup>-1</sup> day<sup>-1</sup> and NPK 100:40:140 g vine<sup>-1</sup> recorded the highest yield (2.69 kg vine<sup>-1</sup>) at Sirsi.

Application of inorganic N 75% + *Azospirillum* 50 g + FYM 10 kg recorded the highest yield of 6.41 kg vine<sup>-1</sup> in black pepper at Sirsi. In an organic farming experiment on black pepper, application of FYM 10 kg + burnt earth 10 kg vine<sup>-1</sup> has given the highest yield at Sirsi. In cardamom highest yield (490 g plant<sup>-1</sup>) was recorded with the application of *Azospirillum* 50 g + FYM 10 kg. The yield of ginger, turmeric, coriander, cumin and fennel was increased by application of inorganic nitrogen in combination with *Azospirillum* and FYM.

Application of two sprays of ferrous sulphate 1% (at 45 and 55 days after sowing) and soil application of zinc sulphate @ 10 kg ha<sup>-1</sup> increased the yield in ginger at Dholi. At Kumarganj, maximum seed yield of coriander (20.03 q ha<sup>-1</sup>) was obtained with soil application of copper sulphate @ 12.5 kg ha<sup>-1</sup> and foliar spray @ 0.25%.

Soft wood grafting was successful in nutmeg at Dapoli with a maximum success of 68% in January.

#### *Crop protection*

Solarized soil fortified with *T. harzianum* and VAM recorded minimum incidence of *Phytophthora* disease in black pepper. Application of Metalaxyl Gold MZ and *T. harzianum* in combination was found to be highly effective in controlling the foot rot

disease of black pepper at Pampadumpara and Sirsi centres.

Ginger rhizomes treated with hot water at 51 °C for 10 minutes and soil application of *T. harzianum* mixed with neem cake resulted in minimum incidence of rhizome rot disease with maximum yield at Kumarganj. The loss due to fungal diseases in ginger was low (13.0%) in rhizomes treated with SAAF + *T. harzianum* in storage with highest recovery (84.5%) of healthy rhizomes. Foliar diseases like leaf spot and leaf blotch in turmeric could be effectively controlled by application of mancozeb + carbendazim as seed treatment and foliar spray.

*T. harzianum* as seed treatment and soil application was effective for the management of wilt disease in coriander. *T. harzianum* with and without carbendazim and neem cake as soil application reduced the incidence of wilt in cumin at Jobner. Soil and seed treatment with *T. harzianum* was also found significantly effective for both wilt and blight diseases at Jagudan. Carbendazim as seed treatment alone or along with soil drenching proved highly effective against root rot in fenugreek.

The population of root grubs in cardamom was reduced significantly with the application of carbofuran (100 g plant<sup>-1</sup>) followed by imidacloprid 0.5 ml l<sup>-1</sup>. Two sprays of monocrotophos and acephate were effective for the control of aphids in cumin.

# कार्यकारी सारांश

## जननिक संसाधन

### काली मिर्च

इडुक्की, कोट्टियूर(केरल), और कूर्ग जिला (कर्नाटक) से काली मिर्च के 51 अक्सशनों को संचित किया। चेताली लोकल, अच्छे फल सज्जायुक्त 17-23 से. मीटर लंबे स्पाइकवाले एक स्थानीय लैंड्रेस, बहु शाखाओंवाले स्पाइकयुक्त एक स्थानीय कृषिजोपजाति, दृढ़ सजावट के कुछ जंगली अक्सशनों और तीक्ष्ण गंध के फलोंयुक्त अक्सशनों आदि सर्वेक्षण काल में संचित कुछ प्रधान संग्रह होते हैं। एक विजातीय स्पीसीस जो पाइपर कोलुब्रिनम के समान होते हैं लेकिन सीधे स्पाइकवाले हैं गुजरात कृषि विश्वविद्यालय, आनन्द( गुजरात) से संचित किया।

### इलायची

अमोमम सुबुलाटम के तीन अक्सशनों और अलपीनिया स्पीसीस का एक अक्सशन सिक्किम से संचित किया।

### वृक्ष मसाले

विट्टल, पुत्तूर, सेदियापुर पुदुवेभा और मूडुपुद्रि (कर्नाटक) आदि जगहों में सर्वेक्षण करने के बाद *गासीनिया इन्डिका* के ग्यारह अक्सशनों को एकत्र किया और *गासीनिया गम्मिगट्टा* के एक अक्सशन को चेम्पानोड (केरल) से एकत्र करके जर्मप्लासम में जोड़ दिया। वृक्ष मसालों के रक्षागृह में *सिनमोमम स्पीसीस*, *मिरिस्टिका स्पीसीस*, *सिसिजियम स्पीसीस* और *गासीनिया स्पीसीस* के क्रमशः 402, 482, 233 और 61 अक्सशनों के अलावा आलस्पाइस के 180 वृक्ष भी होते हैं।

### वैनिला

वैनिला प्लानिफोलिया के 30 संग्रह जिसमें बहुशाखाओंवाले पुष्पक्रम का एक संग्रह भी शामिल है जिसके द्वारा वैनिला के कुल अक्सशनों को 73 तक बना दिया। सफेद फूलोंवाले एक भिन्न प्रकार भी *वी. अन्डामानिका* में देख लिया।

## पत्रिका

पत्रिका के 7 अक्सशनों को भारतीय कृषि अनुसंधान संस्थान का क्षेत्रीय स्टेशन, कट्टैन ( हिमाचल प्रदेश) से संग्रह करके मौजूदा जर्मप्लासम में जोड़ दिया और ऐसे उनमें कुल 47 अक्सशनों बना दिये।

## इन विट्रो जीन बैंक

काली मिर्च के 10 अक्सशनों, इलायची के 10 अक्सशनों, अदरक के 36 अक्सशनों तथा हल्दी के 27 अक्सशनों को इन विट्रो जीन बैंक में जोड़कर कुल 750 अक्सशनों द्वारा उसे मजबूत बना दिया। पी.बारबरी, एक आपत्कारी स्पीसीस और वी प्लानिफोलिया का परिसंपुटन काचन तरीके द्वारा क्रयोप्रिसरवेशन क्रमशः 70% और 80% सफलता के साथ मानकीकृत किया। इलायची के प्ररोहाग्र की काचन रीति द्वारा 80% क्रयोप्रिसरव किये प्ररोह के नोक की प्राप्ति के साथ क्रयोप्रिसरवेशन को मानकीकृत किया।

## जर्मप्लासम का चरित्र चित्रण

### काली मिर्च

काली मिर्च के एक सौ दस अक्सशनों को IPGRI विवरण के आधार पर चरित्रांकित किया। जर्मप्लासम अक्सशन का संग्रह 1041 (आई सी -316598) को राष्ट्रीय पादप जननिक संसाधन ब्यूरो, नई दिल्ली के साथ अपने खुर गलन रोग के प्रति खेत सहायता के लिए पंजीकृत किया।

इन्टर सिमिल सीक्वन्स रिपीट (ISSR) - PCR को पाइपर के विभिन्न विश्लेषणों में सफल रूप से प्रयोग करता है। सात पाइपर स्पीसीस जैसे पी नाइग्रम, पी लॉगम, पी छाबा, पी ब्राकिस्टाक्युम, पी बाबाबुडानी, पी ट्राइकास्टाक्योन और पी कोलुब्रिनम को सिंगिल आई एस एस आर प्राइमर का प्रयोग करके अलग किया जाता है। काली मिर्च के कृष्ट संकरों की पहचान के लिए मूल पौधों के विशिष्ट अंककों की पहचान हेतु

ISSR-PCR को सफल रूप से प्रयुक्त किया गया। काली मिर्च में गुणवत्ता स्वभाव से संबन्धित जीन की पहचान के लिए तुलनात्मक जीन अभिव्यक्ति विश्लेषण चालू किया। श्रीकरा किस्म के किशोर पौधों तथा स्पाइकिंग पौधों के पत्तों से नवीन GTC तरीके द्वारा DDRT-PCR (विभेदीय प्रदर्शन) के आधार पर RNA वियुक्त किया। चयन किये अनियमित 13 मेर प्राइमर और ओलिगोड (T)18 के साथ प्रवर्धन प्रतिक्रिया में विभिन्न जीन के उतार - चढ़ाव नियामक का निरीक्षण किया।

### इलायची

इलायची के उनचास अक्सशनों को IPGRI विवरण के आधार पर चरित्रांकित एवं सूचीबद्ध किया। इलायची के 114 अक्सशनों में पर्ण चित्ती और राइजोम गलन का आपतन अंकित किया और 1 उच्च प्रतिरोधी, 14 प्रतिरोधी, 54 सह्यतायुक्त, 35 सुप्रभाव्य और 10 अधिक सुप्रभाव्य अक्सशनों की पहचान की गयी। खड़े, अर्ध खड़े पुष्प गुच्छ और अरोमिल पर्ण स्वभावयुक्त अक्सशनों पर्ण चित्ती रोग के प्रतिरोधी होता है और जो भूशायी और संयुक्त पुष्प गुच्छवाले होता है राइजोम गलन के सुप्रभाव्य होता है। चयन किये इलायची के सौ से अधिक अक्सशनों को RAPD, ISSR और PCR-RFLP प्रोफाइलों द्वारा चरित्रांकित किया।

### अदरक एवं हल्दी

हल्दी के 96 अक्सशनों/किस्मों में 15 अनियमित डिकेमर प्राइमर्स तथा अदरक के 96 अक्सशनों/ किस्मों में 10 अनियमित डिकेमर प्राइमर्स द्वारा RAPD प्रोफाइलिंग करने पर हल्दी किस्मों/ अक्सशनों के बीच अच्छा बहुरूप तथा अदरक में कम बहुरूप सूचित किया। हल्दी अक्सशन 657(IC- 296550) NBPGR, नई दिल्ली द्वारा अधिक कुरकुमिन और अच्छी उपजता के लिए पंजीकृत किया गया।

### फसल सुधार

#### काली मिर्च

काली मिर्च की चार प्रजातियाँ जैसे OPKm(पूर्व पक्वता, उच्च उपजता, घने फल, लंबे स्पाइक और सूखा सह्यता), HP-728(पूर्व पक्वता और उच्च उपजता), HP-780(उच्च उपजता और उच्च ड्रयेज) और HP-1411(उच्च उपजता) मूल्यांकन की अंतिम दशा पर पहुँच रही है और किसानों के खेत में लगातार अच्छी निष्पत्ति प्रस्तुत करती है।

काली मिर्च के परपरागित एवं स्वपरागित संततियों की संख्या के द्विसंयोजन और वंशागति प्रतिरूप की विषम युग्मता की प्रकृति और विस्तार समझने के लिए अध्ययन किया गया। काली मिर्च के आणविक मानचित्र विकसित करने के लिए RAPD अंककों का प्रयोग करके प्राथमिक डेटा पैदा किया।

काली मिर्च के एप्रोबेक्टोरियम उपचार किये बीजपत्राधर कर्तौत्तक को कानामाईसिन चयन माध्यम में रखकर पौधों को उत्पादित किया। ओस्मोटिन और B-1,3 ग्लूकानेस होनेवाले प्यूटेटीव ट्रान्सजेनिक पौधों को प्राप्त हुआ। मातृ पौधों में ग्लूकानेस और ओस्मोटिन दोनों की स्थिरता के प्रकट का पता लगाने के लिए अध्ययन प्रगति पर रहे है। प्यूटेटीव कार्टिनेस और फाइटोफथोरा प्रतिरोधी जीन के प्रवर्धन के लिए जीन विशिष्ट प्राइमर्स का प्रयोग किया जाता है। उनके cDNAs के 3' अन्त का अनुक्रम बनाया।

#### इलायची

चयन किये विशिष्ट स्वभाववाले ( उच्च उपजता, उच्च गुणवत्ता, सूखा सह्यता, राइजोम गलन सह्य तथा पर्ण चित्ती और मोसाइक रोग के प्रतिरोधी) 6 मातृ पौधों का प्रयोग करके इलायची के 19 संयोजन में संकरण चालू किया और उनके फल सज्जा के प्रतिशत में 22% से 66% तक अन्तर होता है।

#### हल्दी

विपणन के लिए तैयार किये हल्दी चूर्ण से DNA वियुक्ति के लिए एक प्रोटोकॉल मानकीकृत किया।

#### वृक्ष मसाले

पेरुवनामुषि में जायफल के संतति मूल्यांकन प्रयोग में अन्य प्रजातियों की अपेक्षा A9-20 और A9-53 जैसी श्रेष्ठ प्रजातियाँ अच्छा निष्पादन प्रस्तुत किया। अष्पंगला में किये कैसिया के संतति मूल्यांकन प्रयोग में श्रेष्ठ किस्म B-3 अन्य प्रविष्टियों की अपेक्षा उच्चतम देखा गया।

जायफल का उत्पादन बढ़ाने हेतु कलम बांधने के लिए मूल स्रोत के रूप में *मिरिस्टिका* के विभिन्न स्पीसीसों के मूल्यांकन करने पर रोपण के तीसरे साल में अन्य की अपेक्षा एम. मलबारिका की दक्षता उच्चतम देखा गया। एम. माग्निफिका वार. फट्टा पर इन्टरस्टॉक के रूप में एम बडोमी को लगाने पर एम फ्राग्रन्स का

कलम बाँधना सफल हो गया और पांच साल के कलम पुष्पित होने और फल देने लगते हैं। सिजिजियम हेयन्यियानम ( लौंग के साथ संगत मूल स्रोत) के क्लॉन सामग्री का गुणन किया और लौंग को कलम बनाकर अतिरिक्त मूल्यांकन के लिए कलम बांध दिया। बौनापन पर द्वि मूल स्रोत के प्रभाव का अध्ययन करने और लौंग की उपजता बढ़ाने के लिए लौंग को एस. कार्याफिल्लाटम (एक बुशी स्पीसीस) पर एस. हेयन्युवानम को इन्टरस्टॉक के रूप में लेकर सफल रूप से कलम बांध दिया।

### वैनिला

अक्तूबर में वी. अन्डमानिका अक्सशनों के काट छांट करने पर एक अक्सशन में अनेक पुष्प बनते देखा और अन्य तीनों पहली बार पुष्पित करने के लिए प्रेरित किया। वी. अन्डमानिका के सफेद और पर्पल फूलवाले अक्सशनों में अन्तर प्रजातीय संकरण और स्व परागण किया गया और फल सज्जा प्राप्त किया। वी. अन्डमानिका और वी. अफिल्ला के बीच सफल अन्तर्विशिष्ट संकरण किया गया।

BAP युक्त विभिन्न संवर्धन माध्यम में 40 दिन के अन्दर वी. प्लानिफोलिया बीजों को अंकुरित देखे गये। फिर भी वृद्धि नियामक रहित माध्यम में अंकुरण करने पर BAP युक्त माध्यम की अपेक्षा साधारण प्रोटोकॉम्स (protocorns) अधिक प्राप्त होने के साथ जड़ रचना सरल बन गये। लगभग 500 प्रोटोकॉम्स वृद्धि की विभिन्न दशाओं पर है और 25 बीजी संततियाँ एक्स विट्रो में स्थापित किया।

### पप्रिका

पप्रिका के पौधों की उपजता 123 से 746 ग्राम है जिसका एक मध्यम मान प्रति पौधे 416 ग्राम है और जिसका विचरण गुणांक 6.51% होता है।

### उच्च उपजवाली किस्में

काली मिर्च की चार प्रजातियाँ आई आई एस आर तेवम (तेवमुंडी का एक चयन जो खुर गलन रोग के प्रति खेत सह्यता और उच्च उपजता दोनों गुणवाले होते हैं), आई आई एस आर गिरिमुंडा (उच्च तुंगता के क्षेत्रों के लिए उचित एक संकरज), आई आई एस आर मलबार एक्सल (ओलिओरसिन अधिक होनेवाले एक संकरज) और आई आई एस आर शक्ति (पेराम्ब्रामुंडा के ओ पी संतति, जो खुर गलन रोग के सह्यता के साथ उच्च उपजता और उच्च ड्रयेज युक्त होता है) और हल्दी की दो प्रजातियाँ जैसे आई

आई एस आर आलप्पी सुप्रोम (आलप्पी फिंगर टरमरिक का एक क्लॉन चयन) और आई आई एस आर केदारम (एक जर्मप्लासम चयन, कुरकुमिन की अधिक मात्रा तथा पर्ण दाग की सह्यता युक्त) निकालने का प्रस्ताव किया।

### गुणवत्ता मूल्यांकन

#### इलायची

इलायची के 77 अक्सशनों का मूल्यांकन करने पर उनमें APG-246, 248, 352 और 378 में 8% से अधिक तेल पाया। APG 357 और 365 में 42%  $\alpha$  टरपिनिल एसिटेट होते देखा जिसमें सिनिओल (Cineole) का अंश अपेक्षाकृत कम होता है।

#### हल्दी

हल्दी के 400 अक्सशनों का मूल्यांकन करने पर अक्सशन 103, 114, 547 और 575 में 5% से अधिक वाष्पशील तेल होता है। अक्सशन 103 और 547 में 16.5% ओलिओरसिन होता है; अक्सशन 295 और 544 में 14% ओलिओरसिन और अक्सशन 240, 592 और 593 में 5.5% कुरकुमिन होता है।

फिनैल अलनिन अमोनिया लाइस को हल्दी में कुरकुमिन जैवसंश्लेषण में दर सीमक एनजाइम के रूप में देखा जाता है। इस एनजाइम को माइक्रोसोमल अंश के साथ संयोजित होते देख लिया। 14 C फिनैल - अलनिन के प्रयोग द्वारा किये समावेशन अध्ययन के आधार पर जैवसंश्लेषण मार्ग में प्रारंभिक पूर्वगामी को फिनैल - अलनिन के रूप में पुष्टि की गयी।

#### कैसिया

सिन्नमोमम बर्मानी (पैदांग कैसिया) के छाल में 2.8% तेल और 11.8% ओलिओरसिन होता है और सी. लाउरेरी (वियतनाम कैसिया) में 3.3% तेल होता है।

#### पप्रिका

पप्रिका अक्सशनों का रंग मूल्य में 39 से 277 ASTA एकक का अन्तर होता है जिसका मध्यम मान 184 ASTA एकक है और परिवर्तन का गुणांक 2.46% होता है।

#### मिश्रित फसलन रीतियाँ

सुपारी बागों में पी. छाबा (प्रति हेक्टर ग्लिरिसिडिया टेक पर लगभग 4000 पौधे) को अन्तराफसल बनाने पर प्रति हेक्टर से 32,400 रुपये अतिरिक्त आमदनी प्राप्त होती है। इसके अलावा टेक वृक्ष से मिट्टी का पारिस्थितिक नइट्रोजन अधिक होता है

और इसके साथ साथ इन पेड़ों के हरे पत्ते पलवार के लिए प्रयुक्त करते हैं।

### एकीकृत पादप पोषण प्रबन्धन

अदरक के लिए मिट्टी में सिंक लगाने पर मिट्टी में सिंक की मात्रा बहुत बढ़ गयी और प्रति हेक्टर 15 कि. ग्राम के दर में सिंक लगाने पर उच्चतम गाढापन प्राप्त हुआ। एक घनीय नमूना मध्यम राइजोम उपजता और उर्वरक लगाने की मात्रा के बीच के संबंध को संतोषपरक दृष्टि से व्यक्त किया। अधिकतम राइजोम उपजता प्राप्त करने के लिए प्रति हेक्टर 6 कि. ग्राम की मात्रा में सिंक उर्वरक लगाना और अधिक राइजोम उपजता प्राप्त होने के लिए प्रति कि. ग्राम 3.4 मि ग्राम अधिकतम मात्रा में DTPA-Zn मिट्टी में देना आदर्श रूप से पर्याप्त हो गया।

### सूखापन का प्रबन्धन

#### इलायची

इलायची के पचानबे जीनरूपों को उनमें सम्मिलित संबन्धित जल मात्रा, विशिष्ट पणों की बोझ और रंध्री गणना के लिए छानबीन किया। अध्ययन किये सभी चरित्रों में महत्वपूर्ण अन्तर अंकित किया। सूखा सह्यता के अतिरिक्त मूल्यांकन के लिए कोडगु जिला में किसानों के खेतों से उच्च बयोमास (biomass) और उपजतावाले तीन संग्रहों को संचित किया।

काफी और इलायची बागों में कांटूर स्टेगेर्ड ट्रेंचस (contour staggered trenches) रखने से मिट्टी एवं जल परिरक्षण में अच्छा प्रभाव देखा गया। ग्रीष्मकाल में 12 दिन के अन्तर एक बार छः घंटे तक छिड़ककर सिंचाई करने से इलायची में 15 दिन के अन्तर सिंचाई करने की अपेक्षा कैप्सूल सेटिंग (capsule setting) बीजकोष सजावट और उपज में वृद्धि होती है।

### रोग प्रबन्धन

#### काली मिर्च

#### फाइटोफथोरा खुर गलन

काली मिर्च की लोकप्रिय प्रजातियों (पत्रियूर 1, शुभकरा और आई आई एस आर शक्ति) के स्वसेचन तथा सस्य संबन्धी उच्चतम दो प्रजातियों (पत्रियूर 1 और शुभकरा) का संकरण

आदि द्वारा बीजीय प्रजातियों को बढ़ा दिया। स्वसेचित प्रजातियों में आई आई एस आर शक्ति, शुभकरा और पत्रियूर-1 प्रजातियों में क्रमशः 13.85%, 9.70% और 7.20% प्रतिरोधक प्रतिक्रिया दिखाई देती है। काली मिर्च के दो सुप्राह्य प्रजातियाँ पत्रियूर - 1 और शुभकरा के संकरण करके मिले 169 संततियों में 7.15% प्रतिक्रिया प्रतिरोधकता दिखा दी।

पी कोलुब्रिनम (फाइटोफथोरा केप्सीसी के प्रतिरोधक) पर बनाये काली मिर्च कलम की दक्षता दल दल क्षेत्रों में सन्तोषजनक होता है। प्रत्येक टेक वृक्षों (सुपारी) के चारों ओर अधिकतम संरक्षण और उपज प्राप्त करने के लिए 5 कलम लगाने की सिफारिश की गयी।

राडोफोलस सिमिलिस और मेलोयिडोगिने इनकोग्निटा दोनों को दमन कराने लायक रिजोबैक्टीरिया की आशाजनक वियुक्तियों को पी काप्सीसी के प्रति छान बीन किया। आई आई एस आर-658, आई आई एस आर -853 और आई आई एस आर -869 आदि वियुक्तियाँ प्रयोगशाला में पी काप्सीसी के प्रतिरोधक बन गये। ये तीनों वियुक्तियाँ फॉसफोरस विलेयकारी के दक्ष देखे गये।

#### वृद्धि रोध रोग(Stunt disease)

कर्नाटक के दक्षिण कन्नडा, मडिकेरी, उडुप्पी और उत्तर कन्नडा जिलाओं तथा केरल के इडुक्की, कन्नूर, कासरगोड, कोषिकोड और वयनाडु जिलाओं के काली मिर्च बागों में वृद्धि रोध रोग प्रभाव का सर्वेक्षण किया। कर्नाटक की अपेक्षा केरल में इस रोग की व्याप्ति और प्रभाव अधिक होता है। इडुक्की और वयनाडु जिलाओं के उच्च तुंगता में स्थित काली मिर्च रोपण में इस रोग का अधिक प्रभाव ध्यान पर आया।

वृद्धिरोध रोग बाधित काली मिर्च के सिरमीय (serological) विश्लेषण करने पर उनमें कुकुम्बर मोसाइक वाइरस (CMV) और बेडनावाइरस (badnavirus) जैसे दो वाइरसों का घनिष्ठ संबंध सूचित करता है। फेरीसिया विर्गैटा मीली बग को बेडनावाइरस संचारण रोगवाहक के रूप में पहचान किया।

विभिन्न क्षेत्रों से संचित खेत नमूनों में CMV बाधा का पता लगाने के लिए डबल एन्टीबोडी सैंडविच एनजाइम लिंक्ड इम्यूनोसोरोबन्ट एस्से (double antibody sandwich enzyme linked immunosorbent assay -DAS-ELISA) तरीका मानकीकृत किया।

### स्पाइक झड़ना (Spike shedding)

विभिन्न फसल संयोजन, निवेश प्रबन्धन और लैट प्रोफाइल में किये निरीक्षण में यह अंकित किया जाता है कि विभिन्न खेत दशाओं में स्पाइक झड़ने में 9% से 87% तक अन्तर होता है। उच्चतम स्पाइक झड़न वर्षा प्रधान और अधिक छायेदार बागों में देखा गया।

आन्त्राकनोस रोग (कोलेटोटोटाइकम ग्लोयियोस्पोरियोयिड्स के कारण होता है), स्त्री फूलों का प्राधान्य, वर्ष प्रधान क्षेत्रों में परागण का अभाव, अधिक छाया और स्पाइक निर्गमन में देरी आदि को स्पाइक झड़ने का प्रमुख कारण पहचान गया। पत्रियूर -1 में स्पाइक संक्रमण 13.0% से 83.4% तक होता है और उच्चतम संक्रमण अधिक छायेदार जगह में उगाए पौधों पर अंकित किया।

पत्रियूर -5, पंचमी और शुभकरा प्रजातियाँ आन्त्राकनोस बाधा के खेत सह्य (field tolerance) देखे गये और पत्रियूर-1 और पत्रियूर-3 अधिक सुग्राह्य (susceptible) हो गये। 14 कृष्ट (cultivars) के बीच चोमला, तेवनमुंडी, करिमुंडा, चेताली चयन, अधिम्बीरियन और अरकलमुंडा आन्त्राकनोस के खेत सह्य देखे गये।

काली मिर्च बेलों के लिए 5-7 दिनों के अन्तर 4-5 बार 40-50 लिटर / पौधे के दर में 22 मार्च से सिंचाई करना, गंदली दशाओं में न्यूनतम 7,500-10,000 लक्स प्रकाश प्रदान करने के लिए टेक वृक्षों की छाया का नियामन करना उच्च तुंगता में स्पाइक झड़ने को नियन्त्रित करने के लिए पर्याप्त होता है। उच्च तुंगता में स्पाइक झड़ने और आन्त्राकनोस के साकल्यवादी प्रबन्धन के लिए सिंचाई के साथ संस्तुत मात्रा में फाइटोसानिटरीस (phytosanitarities), रोग रोधक और पोषण प्रबन्धन रीतियाँ आवश्यक हो जाता है।

### अदरक

#### जीवाणुक म्लानी (Bacterial wilt)

मिट्टी और अदरक राइजोम में *रालस्टोनिया सोलानसीयरम* का पता लगाने के लिए DAS-ELISA मानकीकृत किया। इस तरीके से मिट्टी और अदरक के परित्वक् से *रालस्टोनिया सोलानसीयरम* का पता नहीं लगा दिया जा सकता है, यह सूचित किया जाता है कि यह रोग जनक जीवाणुक म्लानी बाधित

अदरक पौधों के मलबा में रह नहीं सकता, जिसका ग्रीन हाउस में बयोएस्से द्वारा आगे पुष्टि की गयी।

*रालस्टोनिया सोलानसीयरम* के आणविक चरित्रांकन के लिए विस्तृत रिबोसोमल DNA प्रतिबन्धन विश्लेषण (Amplified Ribosomal DNA Restriction Analysis - ARDRA) प्रविधि मानकीकृत किया। *रालस्टोनिया* के बयोवर्स (biovars) को Sau IA और MSP I प्रतिबन्धन एनजाइम के साथ ARDRA का प्रयोग करके भिन्न किया जा सकता है। *रालस्टोनिया सोलानसीयरम* की 33 वियुक्तियों का Rep-PCR और ITS-PCR द्वारा आणविक विश्लेषण करने पर अदरक के जीवाणुक म्लानी का कारक *रालस्टोनिया* के एकमात्र विषाक्त वंश परंपरा का अस्तित्व प्रकट हुआ।

*टी विरिडे* के कोशरहित संवर्धन निस्यंद (cell free culture filtrate) के प्रतिजीवाणु क्रियाएँ *रालस्टोनिया सोलानसीयरम* के प्रति प्रभावी देखा गया। संवर्धन निस्यंद इन *विट्रो* रोगजनक की वृद्धि और गुणन के प्रति सक्रिय हो गया। यह संवर्धन निस्यंद 70-90 °C के ताप द्वारा असक्रिय बनाया गया। *टी विरिडे* के उत्परिवर्ती व्युत्पन्न (mutant derivative (albino)) प्रतिजीवाणुक उपापचय (antibiotic metabolites) का उत्पादन नहीं करता है।

अदरक राइजोम को आर. सोलानसीयरम विशिष्ट रोगप्रतिकारकों को (DAS-ELISA) लगाने से आर. सोलानसीयरम बाधा रहित में राइजोम सोलाराइसेशन का सफल प्रभाव साबित किया। राइजोम सोलाराइसेशन के द्वारा कीटबाधा रहित बनाये बीजों को खेत में लगाने पर जीवाणुक म्लानी का नियन्त्रण करने में प्रभावी देखता है।

अदरक को रोग से निभानेवाले एन्डोफाइटिक (Endophytic) बैक्टीरिया को अदरक की रोगग्रस्त राइजोम तथा फ्यूडोस्टम से पाया गया।

#### मृदु गलन

*पितियम स्पीसीस* द्वारा आनेवाली अदरक के मृदु गलन रोग की प्रतिक्रिया के लिए अदरक के 200 अक्सशनों का छान बीन किया। अक्सशन 130 रोग के सह्य देखे गये।

पौध वृद्धि सहायक रिजोबैक्टीरिया (PGPR) के विभिन्न वियुक्तियों

के साथ अध्ययन करने पर अदरक के मृदु गलन रोग के प्रति PGPR विद्युक्तियों में आई आई एस आर 13, आई आई एस आर 51, आई आई एस आर 151 आई आई एस आर 152 और आई आई एस आर 906 रोग आपतन को 5% से कम बनाने में सक्षम बन गये। मेटालक्सिल (रिडोमिल MZ -72wp) 0.125% के साथ बीज उपचार तथा नीम केक के साथ इसे दो बार मिट्टी में लगाने पर रोग आपतन 10% से कम हो गये।

### वैनिला

केरल के कालिकट, इडुक्कि और वयनाडु जिलाओं में व्याप्त 22 वैनिला बागों तथा कर्नाटक के दक्षिण कन्नडा, उडुप्पि और उत्तर कन्नडा जिलाओं में रोग सर्वेक्षण चालू किया। सर्वेक्षण किये अधिकांश बागों में मोसाइक और उतकक्षय (necrosis) जैसे दो वाइरल रोगों का निरीक्षण किया और इन रोग आपतनों में 0 से 10% तक अन्तर होता है। कम उँचाई (<100 मीटर) के लगभग सभी वैनिला बागों में *स्लोयियोस्पोरियोथिड्स* के कारण आनेवाले रोग और जिसके द्वारा अपक्व पीलापन और बीन झडना देखा गया। रोग बाधा के लिए उच्च तापमान (>30°C) और निम्न सापेक्ष आर्द्रता (<60%) को पहले से ही प्रवृत्त कारक के रूप में पहचान की गयी।

### नेमटोड प्रबन्धन

अदरक एवं हल्दी दोनों के 50 जर्मप्लासम अक्सशनों को *एम. इनकोग्निटा* के प्रति छानबीन किया। इन में दूसरी छान बीन के लिए 14 अदरक एवं 13 हल्दी अक्सशनों की सूची बनायी। नेमटोड प्रतिरोधक अक्सशनों जैसे अक्सशन 202 अदरक में तथा अक्सशन 82 और अक्सशन 84 हल्दी में उपजता और गुणवत्ता स्वभाव के लिए उच्चतम देखा गया। काली मिर्च के 9 जर्मप्लासम अक्सशनों की प्रतिरोधकता की पुष्टि करने के लिए पेरुवन्नामुषि फार्म में इन सिटु परीक्षणआत्मक छानबीन लगा दिया।

### पी जी पी आर विशेषीकरण

काली मिर्च बागों से 103 एन्डोफाइटिक बैक्टीरिया को विभिन्न प्रस्तावों द्वारा विद्युक्त किया। इन्हीं विद्युक्तियों को कॉलनी आकारिकी, प्रतिजैविक सूक्ष्मग्राहिता, काली मिर्च पौधों को उपनिवेशित करने की उनकी क्षमता और नेमटिसिडल विशेषता आदि द्वारा चित्रांकित किया। इन्हीं विद्युक्तियों का नेमटिसिडल कार्यक्षमता में

शून्य से 29% तक का अन्तर होता है। जीन का जैव नियन्त्रण हेतु विशिष्ट प्राइमर विकसित करने के लिए इन *सिलिको* (In silico) विश्लेषण पूरा किया। जीवित और मरे हुए नेमटोडों को भिन्न करने के लिए एक असाधारण रीति विकसित की। काली मिर्च, इलायची, अदरक आदि से विद्युक्त किये नेमटिसिडल रिजोबैक्टीरिया के जीनोमिक (Genomic) DNA को CTAB-SDS रीति से विद्युक्त किया और ARDRA फिंगर प्रिंटिंग के लिए प्रक्रिया मानकीकृत की।

पी जी पी आर, आई आई एस आर-522, आई आई एस आर-528 और आई आई एस आर 658 पौध परजीवी नेमटोड का दमन किया और काली मिर्च के पीलापन रोग को महत्वपूर्ण रूप से कम कर दिया। अदरक खेत में आई आई एस आर -13, आई आई एस आर -51 और आई आई एस आर 866 लगाने पर उपजता में 31.8% से 56.4% तक वृद्धि होती है। काली मिर्च के चार कवग जैवएजेंट का मूल्यांकन करने पर *वर्टिसिलियम क्लामिडोस्पोरियम* और *स्कोपुलारियोप्सिस स्पीसीस* नेमटोड बाधा को नियन्त्रित करने में प्रभावी देखे गये।

### कीट नाशक जीव का नियन्त्रण

#### पोल्लु बीटल

काली मिर्च जर्मप्लासम रक्षागृह में उपलब्ध 165 कृष्ट अक्सशनों और 42 संकरजों को *पोल्लु बीटल* (*लॉंगिटारसस निग्रिप्रिन्सिस*) के प्रति छान बीन किया।

#### मूल मीली बग

केरल के कासरगोड, कन्नूर, कोषिकोड और इडुक्कि जिलाओं में व्याप्त 54 जगहों के 162 काली मिर्च बागों तथा कर्नाटक के दक्षिण कन्नडा, उडुप्पी, उत्तर कन्नडा और हस्सन जिलाओं के 18 जगहों में स्थित 50 काली मिर्च बागों में काली मिर्च पर मूल मीली बग (*प्लानोकोकस स्पीसीस*) के वितरण अध्ययन के लिए सर्वेक्षण किया। उनमें इडुक्कि जिला के पांच जगहों में स्थित छः बागों तथा कर्नाटक के दो जगहों में स्थित पांच बागों में कीट बाधा दिखाई पडी।

#### अदरक एवं हल्दी

#### अदरक का प्ररोह बेधक

जर्मप्लासम रक्षागृह में उपलब्ध 551 अदरक अक्सशनों को प्ररोह बेधक (*कोनोगीतस पंक्टिफरालिस*) के प्रति छानबीन किया



और उनमें किसी भी अक्सरशनों में 5% से कम प्ररोह पर कीट बाधा नहीं दिखाई पड़ी ; 69 अक्सरशनों में 5-10% प्ररोह कीट बाधित देखे गये।

पिछले साल प्ररोह बेधक के प्रति नीम का तेल और वाणिज्यिक नीम उपज (दोनों 1% गाढता में) जुलाई - अक्तूबर में पाक्षिक अन्तराल में छिडकने पर आशाजनक देखे गये जो इस साल अदरक पर कीट बाधा कम करने में प्रभावी नहीं हो गये।

अदरक के 77 अक्सरशनों तथा हल्दी के 12 अक्सरशनों के सूखे राइजोम को सिगरट बीटल (लासियोडेरमा सेरिकार्न) द्वारा आनेवाली नुकसान के प्रति छान बीन किया और उनमें अदरक के 2 अक्सरशनों का राइजोम कीट बाधा रहित देखे गये।

### फसलोत्तर प्रबन्धन

काली मिर्च सुखाने के लिए विद्युत् चालित सोखता (TNAU नमूना) की दक्षता का मूल्यांकन किया। इसके द्वारा पतले स्तर (2 से.मीटर घनेवाले बेड) में काली मिर्च सुखाने के लिए लगभग 9.0घंटे आवश्यक होता है और गहरे बेड सुखाने (बेड का घना 6.5 से मीटर) के लिए 14.5 घंटे आवश्यक होता है और उसका अंतिम आर्द्रता का अंश 10.7% होता है।

### आर्थिकी

केरल, तमिलनाडु और कर्नाटक में वैनिला खेती पर आयोजित सर्वेक्षण से यह सूचित करता है कि फसल को अच्छा भाव प्रबल करने से वैनिला खेती में द्रुत वृद्धि होती है। खेती का अनुमानित लागत सूचित करता है कि कर्नाटक और केरल में लाभदायक मूल्य का अनुपात क्रमशः 3.3 और 3.8 होता है। कर्नाटक के कूरग जिला में इलायची और काली मिर्च खेती को प्रोत्साहित करने के लिए NATP परियोजना के अन्दर विकसित तकनोलजी को उनकी आर्थिक क्षमता के लिए मूल्यांकित किया।

### तकनोलजी अन्तरण

#### प्रशिक्षण एवं विकास कार्यक्रम

संस्थान ने मसालों का उत्पादन तकनोलजी पर दो प्रशिक्षण कार्यक्रम आयोजित किये और एक कार्यक्रम प्रत्येक ऑन फार्म प्रोसेसिंग ऑफ स्पाइसस और काली मिर्च में वाइरल रोग नियन्त्रण एवं नर्सरी प्रबन्धन पर है जो राज्य कृषि एवं बागवानी विभाग के खेत विस्तार कार्यकर्ताओं और भारतीय कृषि अनुसंधान परिषद

के अधीनस्थ संस्थानों के कृषि विश्व विद्यालयों के शोध कर्मियों के लिए है। स्नातकोत्तर छात्रों के लिए जैवप्रौद्योगिकी / जैवरसायन शास्त्र और जैव सूचना, तथा अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना के वैज्ञानिकों के लिए मसाला अनुसंधान में परीक्षणात्मक योजनाएँ और कम्प्यूटर एप्लिकेशन्स और श्रीलंका के वैज्ञानिक के लिए काली मिर्च की जैव प्रौद्योगिकी और एरिट्रिया से आए प्रतिनिधियों के लिए मसालों की खेती एवं संसाधन पर प्रशिक्षण कार्यक्रम आयोजित किये। संस्थान के वैज्ञानिक, प्रशासनिक एवं तकनीकी स्टाफों को अपने संबन्धित विषयों पर प्रशिक्षण देने के लिए देश के विभिन्न संस्थानों पर नामित किया। इस संस्थान के वैज्ञानिकों के मार्गदर्शन पर विभिन्न विश्व विद्यालयों के स्नातकोत्तर छात्रों द्वारा चालीस ह्रस्व कालीन अनुसंधान परियोजनाएँ चालू कीं। परामर्श संसाधन सेल ने प्रशिक्षण कार्यक्रम, परामर्श सेवाएँ और संस्थान में विकसित तकनोलजी अन्तरण प्रदान किये और 6,86,000 रुपए की आमदनी कमा ली।

### रोपण सामग्रियों का उत्पादन

काली मिर्च के पांच लाख मूल लगाए लैटरल, 25,799 इलायची बीजपौधे, 1,226 इलायची के सकेस अदरक एवं हल्दी दोनों के 2 टन बीज राइजोम, गार्सीनिया के 649 ग्राफ्ट्स, जायफल का 6000 ग्राफ्ट्स आदि उत्पादित करके किसानों और अन्य विकासात्मक एजेंसियों को वितरित किये।

### कृषि तकनोलजी सूचना केन्द्र (ATIC)

मसालों के सुधार किये प्रजातियों की रोपण सामग्रियाँ और ट्राइकोडरमा संवर्धन किसानों एवं अन्य एजेंसियों को कृषि तकनोलजी सूचना केन्द्र(ATIC) के एकद्वार वितरण रीति द्वारा संवितरित किये। 1572 किसानों को मसाला खेती की विभिन्न पहलुओं पर परामर्श सेवाएँ प्रदान किये और विभिन्न स्कूलों से 529 छात्र इस केन्द्र में आये। ATIC ने रोपण सामग्रियाँ , ट्राइकोडरमा संवर्धन, विस्तार पुस्तिकाएँ और मिट्टी और खाद परीक्षण आदि द्वारा 1,48,786 रुपए कमा लिये।

### कृषि विज्ञान केन्द्र

कृषि विज्ञान केन्द्र(KVK) ने किसानों, बेरोजगार युवाओं, स्कूल से छूट गये छात्रों और विस्तार कार्यकर्ताओं के लिए 91 प्रशिक्षण कार्यक्रम आयोजित किये जिसमें 765 स्त्रियों सहित 1754

प्रशिक्षार्थी भाग लिये। 20 शिक्षित, बेरोजगार ग्रामीण महिलाओं के लिए स्वरोजगार प्राप्त करने लायक बांस की हस्तशिल्प बनाने में एक महीने का एक उन्नत प्रशिक्षण कार्यक्रम प्रदान किये। कृषि विज्ञान केन्द्र ने उच्च उपजवाले भिंडी जैसी सब्जी (okra) (अरका अनामिका प्रजाति) उच्च उपजवाले लोबिया (अरका गरिमा और शरिका) और कुट्टनाडु अंडे देनेवाली बतख पर किसानों के खेत में महत्वपूर्ण प्रदर्शन आयोजित किये और जिसका फल आशाप्रद होता है। करेला में फल मक्खी प्रबन्धन, अनूप मृदावाले क्षेत्रों में कलम बाँधे काली मिर्च का प्रयोग और अमदकाल दुग्ध पशुओं में पोस्टाग्लान्डिन (prostaglandin) F<sub>2</sub> x के प्रभाव का परीक्षण आदि में खेतीगत परीक्षण प्रयोग आयोजित किये।

कृषि विज्ञान केन्द्र ने संस्थान में विकसित कृषि तकनोलजी प्रसरण करने के लिए दो किसान मेला एवं प्रदर्शनी आयोजित किये, रेडियो भाषण और लोकप्रिय लेख प्रकाशित किये। किसानों को विभिन्न अनुसंधान संस्थानों तथा खेतों को दिखाने के लिए पांच शिक्षा दौरा आयोजित किये। पशु चिकित्सालय द्वारा 475 परामर्श / सलाहदारी/ गृह सेवाएँ और 311 कृत्रिम बीजारोपण प्रदान किये। कृषि विज्ञान केन्द्र की मदद से कई ग्रामीण युवाओं ने कृषि पौधाघर, बर्मी कम्पोस्टिंग, फल संसाधन, हस्त शिल्प निर्माण, बकरी पालन आदि स्वरोजगार उप व्यवसाय शुरू किये। कृषि विज्ञान केन्द्र ने मसाले, फल, रोपण फसल, आलंकारिक आदि की रोपण सामग्रियाँ बेचकर 4,06,832 रुपए तथा 22,395 रुपए पशु चिकित्सालय द्वारा कमा लिये। कृषि विज्ञान केन्द्र के विकास वलंटियर वाहिनी (Vikas Volunteer Vahini) क्लब के क्षेत्र में अर्जित उत्कृष्ट सेवा के लिए कृषि एवं ग्रामीण विकास का देशीयकृत बैंक द्वारा प्रशंसा प्रमाणपत्र प्राप्त हुआ।

## सूचना सेवाएँ

### जैवसूचना केन्द्र

जैव सूचना केन्द्र ने कार्षिक जैव सूचनाओं पर दो दिन की कार्यशाला तथा जैवसूचनाएँ और जैवप्रौद्योगिकी -साधन एवं अनुप्रयोग पर 21 दिनों का प्रशिक्षण कार्यक्रम आयोजित किये। इस केन्द्र ने जैवसूचनाओं में पाठ्यक्रम आयोजित करने में इलक्ट्रॉनिक अक्रडिटेशन ऑफ कम्प्यूटर कोर्सस, कालिकट के साथ कार्य किया।

चार डेटा बेसस जैसे *फाइटोफथोरा* सूचना संसाधन : *फाइटोफथोरा*

स्पीसीस के लिए एक व्यापक वेब संसाधन; *कुरकुमा* स्पीसीस ; *मिरिस्टिका* स्पीसीस और जायफल जर्मप्लासम ; इलायची तेल का रासायनिक संघटक और उसके उपापचयी तरीका और दो सेप्टेवर जैसे , फिटफाइंडर (phytfinder) (अपने आकृतिक स्वभाव के आधार पर *फाइटोफथोरा* स्पीसीस की पहचान के लिए) और बयोवरचार (Biovarchar)(आर सोलानसीयरम का बयोवार चरित्रांकन के लिए ) आदि विकसित किये।

### एकीकृत देशीय कृषि संसाधन सूचना रीति (INARIS)

वैनिला के लिए एक अन्तः क्रिया विशेषज्ञ रीति विकसित की और काली मिर्च (काली मिर्च संकलन) के लिए विशेषज्ञ रीति अधुनातन की। मसाला फसलों के लिए एक अंकीय कोड पुस्तक विकसित किया और मसाला डेटाबेस प्रबल बना दिया। मसाला फसलों का जिलातर वितरण पर संकेंद्रित करने के लिए मसालों पर भू डेटाबेस के प्रयोग से एक मसाला मानचित्रावली विकसित की।

### अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना

अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना (AICRPS) देश के सबसे बड़े नेटवर्क है जो 19 समन्वित और 8 स्वैच्छिक केन्द्रों में मसाला अनुसंधान का आयोजन एवं समन्वयन किया जाता है।

### फसल सुधार

अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना केन्द्रों ने मसाला फसलों के जननिक संसाधनों को प्रबल बनाया और अब जर्मप्लासम में काली मिर्च की 650 अक्सशनें, इलायची की 369 अक्सशनें, अदरक की 644 अक्सशनें, हल्दी की 1307 अक्सशनें, वृक्ष मसालों की 228 अक्सशनें और बीजीय मसालों की 3901 अक्सशनें शामिल होते हैं। इस जर्मप्लासम को विविध प्राचलों के लिए मूल्यांकित किया और प्रत्येक फसल में आशाजनक अक्सशनों की पहचान की गयी।

अम्बलवयल में, काली मिर्च की कुछ प्रजातियाँ जैसे पंचमी, पत्रियूर 2, पत्रियूर 3, पत्रियूर - 4 और अक्सशनें 2426 और 2445 आदि केरल के उच्चतर क्षेत्रों के लिए आशाजनक देखा गया। इलायची अक्सशनों जैसे, CL 692, CL-730, और D-237 मुडिगरे में आशाजनक पहचान की गयी और उन्हें प्रजातीय मूल्यांकन प्रयोग में जोड़ दिया गया।

अदरक में, उच्चतम स्वच्छ राइजोम उपजता पोटांगी में V<sub>3</sub>S<sub>1</sub>-8 (28.25 टन/ हेक्टर) में अंकित किया। अदरक अक्सशनों के सूखे उपज मात्राओं में 13.0% से 22.5% तक का अन्तर होता है। ओलिवोरसिन और सुगन्धित तेल की मात्राओं में क्रमशः 4.00% से 9.67% और 0.25% से 2.00% तक का अन्तर होता है और उसमें निहित कच्चा रेशा में 3.93% से 5.95% तक अन्तर होता है। हल्दी में, उच्चतम स्वच्छ राइजोम उपजता पोटांगी में किये प्रारंभिक मूल्यांकन प्रयोग के अन्दर PS-39(26.62 टन/ हेक्टर) में अंकित किया। सोलन में , पांच संग्रह जैसे, ST-365, BDJR-1244, CIs -29, PTSS-24 और DKH-26 ने कन्ट्रोल की तुलना में नियन्त्रण के अधीन 17.64 से 32.62% अधिक उपजता दी।

जीरा में सफल संकरण के लिए, अनखुले कलियों में पूर्वाहन 10 बजे से पहले हल्के गुलाबी रंग होते वक्त विपुंसन चालू करने की सिफारिश की और विपुंसन के बाद 11 बजे से अपराहन 7 बजे के अन्दर परागण अगले दिन या तीसरे दिन या दूसरे और तीसरे दिन (दो बार) करना चाहिए। मेथी अक्सशनों, HM -444 (एक हरे बीज आवरित उत्परिवर्ती) और HM-372 और HM-376,(पीला बीज आवरित उत्परिवर्ती) उच्चतम बीज उपजता क्रमशः 23.9 किंवटल/ हेक्टर 33.85 किंवटल/ हेक्टर और 32.65 किंवटल /हेक्टर दिया जाता है। HM-444 मृदुरोमित मिलड्यू और चूर्णिल मिलड्यू दोनों रोगों के प्रतिरोधक होता है। जोबनर में, RTP-4 उच्चतम उपजता (1518 कि.ग्राम / हेक्टर) अंकित की जिसके पीछे आता है RTP-8(1477 कि.ग्राम /हेक्टर)और RTP-9(1471कि.ग्राम/हेक्टर) और इन्हें CVT के अन्दर मूल्यांकित किया जाएगा। CVT/CYT द्वारा कई प्रजातियों को उपजता और गुणवत्ता प्राप्ति के लिए पहचान किया।

#### फसल प्रबन्धन

सिरसी में काली मिर्च -सुपारी मिश्रित फसलन में 20 लिटर जल/बेल/ दिन और NPK100:40:140 ग्राम/बेल की दर में लगाने पर उच्चतम उपजता (2.69 कि.ग्राम/बेल ) अंकित की। अजैव नाइट्रोजन 75% + अजोस्फिरिल्लम 50ग्राम + FYM10 कि.ग्राम लगाने पर सिरसी में काली मिर्च की उच्चतम उपजता 6.41 कि.ग्राम /बेल अंकित की। काली मिर्च पर जैव

कृषि परीक्षण करने पर FYM 10 कि.ग्राम + तप्त मिट्टी 10 कि.ग्राम / बेल के दर में लगाने पर सिरसी में उच्चतम उपजता प्राप्त हुई। इलायची में अजोस्फिरिल्लम 50 ग्राम + FYM10 कि. ग्राम लगाने पर उच्चतम उपजता (490ग्राम/ पौधा ) अंकित की। अजैव नाइट्रोजन, अजोस्फिरिल्लम और FYM के साथ लगाने पर अदरक, हल्दी, धनिया, जीरा और सौंफ में उपजता बढ गयी।

धोली में फेरस सल्फेट 1% (बोने के 45 और 55 दिन के बाद) दो बार छिडकने तथा सिंक सल्फेट 10 कि.ग्राम/ हेक्टर के दर में मिट्टी में लगाने पर अदरक उपजता में वृद्धि होती है। कुमारगंज में कोप्पर सल्फेट 12.5 कि. ग्राम/हेक्टर के दर में मिट्टी में लगाने तथा 0.25% के दर में पत्तों पर छिडकने से धनिया की अधिकतम बीज उपजता (20.03 किंवटल/ हेक्टर) प्राप्त हुई। धापोली में जायफल का मृदु शाख कलम बांधना जानुवरी में 68% अधिकतम सफल हुई।

#### फसल संरक्षण

काली मिर्च में सोलाराइस्ट मृदा टी. हर्जियानम और VAM के साथ मजबूत करने पर फाइटोफथोरा रोग का न्यूनतम आपतन अंकित किया। पाम्पाडुंपारा और सिरसी केन्द्रों में काली मिर्च के खुर गलन रोग नियन्त्रण करने में मेटालक्सिल गोल्ड (metalaxyl Gold) MZ और टी हर्जियानम मिलाकर लगाने पर उच्चतम प्रभाव देखा जाता है।

कुमारगंज में 51 °C के गरम पानी में अदरक अक्सशनों को 10 मिनट उपचार करने तथा नीम केक और टी हर्जियानम मिश्रण मिट्टी में लगाने पर राइजोम गलन रोग का आपतन कम होने के साथ अधिकतम उपजता भी प्राप्त होते है। संभरण करते वक्त SAAF+ टी हर्जियानम के साथ राइजोम उपचार करने पर कवग रोग के कारण अदरक की हानी बहुत कम (13.0%) होने के साथ स्वस्थ राइजोम की उच्चतम प्राप्ति (84.5%) भी होती है। हल्दी में पर्ण चित्ती और पर्णदाग जैसे पर्ण रोगों को मानकोजेब + कारबनडासिम का बीज उपचार करने तथा पत्तों पर छिडकने से प्रभावी रूप से नियन्त्रित किया जा सकता है।

टी हर्जियानम बीज उपचार के रूप में और मिट्टी में लगाने पर धनिया का प्लानी रोग नियन्त्रित करने में प्रभावी देखे गये। टी हर्जियानम करबनडासिम और नीम केक के साथ या रहित

मिट्टी में लगाने पर जोबनर में जीरा के म्लानी आपतन कम हो गये। टी हर्जियानम के साथ मृदा एवं बीज उपचार करने से जगुदान में म्लानी एवं चित्ती दोनों रोगों में अच्छा प्रभाव देखा गया। कारबन्डासिम केवल बीज उपचार के रूप में या मृदा में लगाने पर मेथी के मूल विगलन के प्रति उच्चतम प्रभाव साबित किया।

कारबोफुरान (100ग्राम/पौधे) और उसके बाद इमिडाक्लोप्रिड (imidacloprid) 0.5 मि. लि / लिटर के रूप में लगाने पर इलायची में मूल खोदक की संख्या बहुत कम कर सकते हैं।

मोनोक्रोटोफोस और असिफेट दो बार छिड़कने से जीरा के एफिड्स का नियन्त्रण करने में प्रभावी देखा गया।

### हिन्दी सेल

राजभाषा कार्यान्वयन समिति की चार बैठकें आयोजित कीं। सितम्बर में हिन्दी सप्ताह मनाया गया। हिन्दी सप्ताह के सन्दर्भ में हिन्दी कार्याशाला आयोजित की। हिन्दी में विभिन्न प्रतियोगिताएँ आयोजित की। हिन्दी में काम करनेवालों के लिए प्रोत्साहन योजना आयोजित की।

# Introduction

## History

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

## Location

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

## Mandate

- To extend services and technologies to conserve genetic resources of spices as well as soil, water and air of spices agroeco-systems.
- To develop high yielding and high quality spice varieties and sustainable production

and protection systems using traditional and non-traditional techniques and novel biotechnological approaches.

- To develop post harvest technologies of spices with emphasis on product development and product diversification for domestic and export purposes.
- To act as a centre for training in research methodology and technology upgradation of spices and to coordinate national research projects.
- To monitor the adoption of new and existing technologies to make sure that research is targeted to the needs of the farming community.
- To serve as a national centre for storage, retrieval and dissemination of technological information on spices.

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

## Organization

The Director is the administrative head of the institute. The Institute Management Committee, Research Advisory Committee and Staff Research Council assist the Director in matters relating to management and research activities of the institute (Fig.1).

Research on various aspects of mandate crops

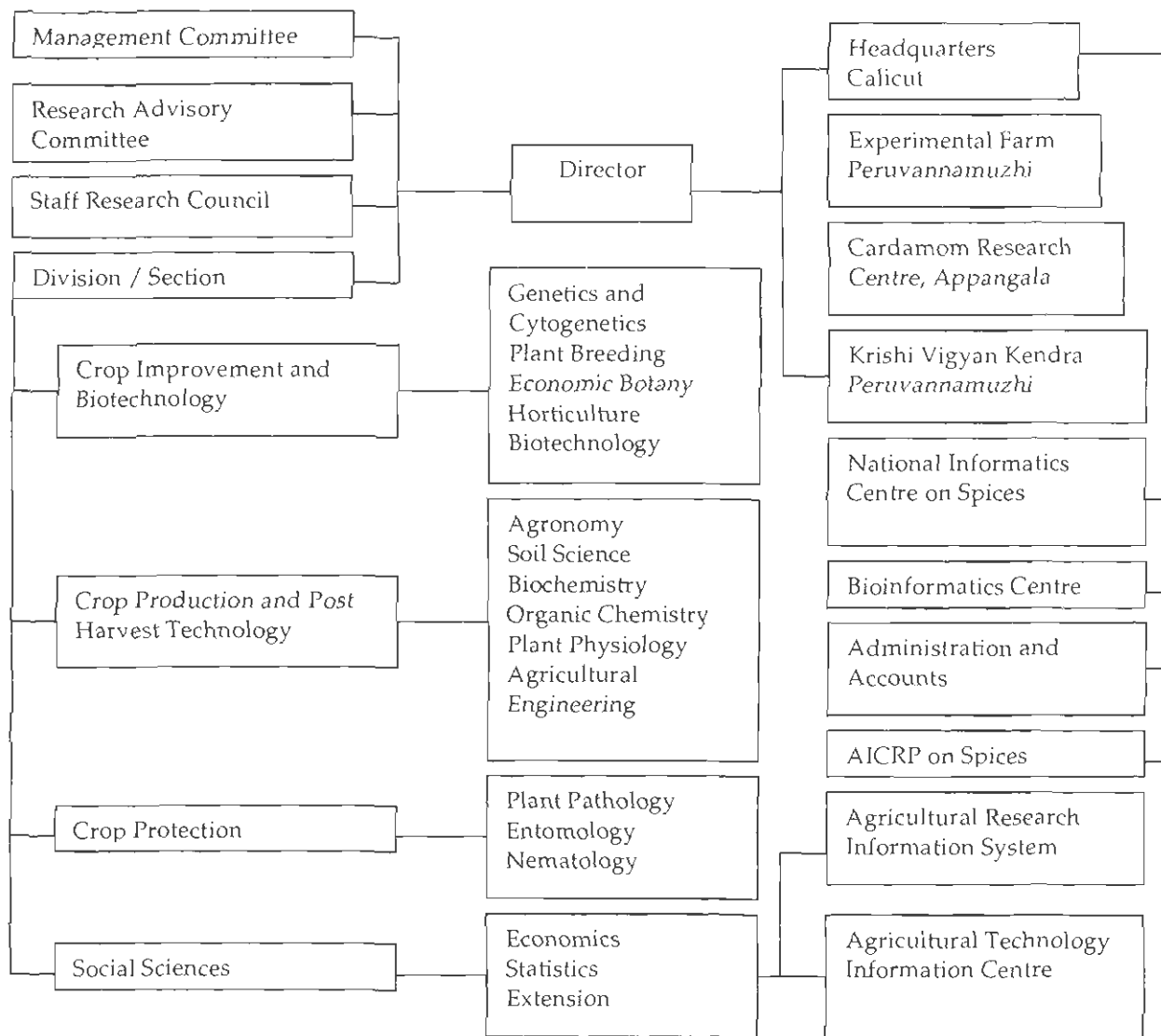


Fig. 1. Organisation of IISR

is conducted in three divisions, namely, Division of Crop Improvement and Biotechnology, Division of Crop Production and Post Harvest Technology and Division of Crop Protection and a Social Sciences Section. The other facilities available at the institute include Agricultural Technology Information Centre, National Information Centre for Spices, Agricultural Research Information System, Bioinformatics Centre and Krishi Vigyan Kendra. The institute also functions as the headquarters of the All India Coordinated Research Project on Spices, and Indian Society for Spices. The institute has

linkages with several universities, research institutes, and developmental agencies for collaborative research and developmental activities in spices.

#### Budget

The total budget of the institute was Rs. 408 lakhs during the year which included Rs. 125 lakhs under Plan and Rs. 283 lakhs under Non Plan and the expenditure under Plan and Non Plan was 125 lakhs and 317 lakhs, respectively. In addition, Rs. 265.23 lakhs was also received as funds from external agencies (Tables 1 and 2).

**Table 1.** Budget of the institute

Particulars	Amount		
	Plan (Lakh Rupees)	Non Plan (Lakh Rupees)	Total (Lakh Rupees)
Establishment	-	228.00	228.00
Traveling allowance	5.00	5.40	10.40
Works	50.00	-	50.00
Other charges	70.00	83.00	153.00
Total receipts	125.00	283.00	408.00
Expenditure	125.00	317.00	442.00

**Table 2.** Funds received from external agencies

Particulars	Amount (Lakh Rupees)
AICRP Spices	151.00
KVK	21.83
ICAR Cess Fund Schemes	14.42
NATP	23.25
DBT schemes	46.49
IPDS	7.74
Pension and gratuity	6.50
Total	265.23

germplasm conservatories and *in vitro* gene banks. The collections include 3299 black pepper, 391 cardamom, 659 ginger, 899 turmeric, 482 nutmeg, 233 clove, 408 cinnamon including cassia, 61 garcinia, 180 allspice, 47 paprika and 73 vanilla accessions. The germplasm was characterized for yield, quality and resistance to pests, diseases and drought. Molecular characterization of germplasm was also initiated.

Various improved varieties with high yield and quality were developed that had a great impact in increasing the production and productivity of spices in the country. Four high yielding and high quality varieties of black pepper, Sreekara, Subhakara, Pournami and Panchami were released, among which Pournami is tolerant to root knot nematode. In cardamom, Suvasini, a high yielding variety suitable for high density planting, Avinash, a variety resistant to rhizome rot disease and Vijetha, a variety resistant to *katte* disease were released. Three ginger varieties with high yield and quality, Varada, Rejatha

## Staff

The institute has a sanctioned strength of 42 scientific, 19 administrative, 37 technical and 63 supporting staff. The staff position of the institute and KVK are given in Tables 3 and 4 respectively.

## Past achievements

Surveys were conducted for collection of germplasm that were conserved in

**Table 3.** Staff position of the institute

Category	Sanctioned	In position			Vacant
		Chelavoor	Peruvannamuzhi	Appangala	
Scientific	42	27	2	3	10
Technical	37	18	13	5	1
Administration	19	15	-	2	2
Supporting	63	27	16	19	1
Total	161	87	31	29	14

Table 4. Staff position of KVK

Category	Sanctioned	In position			Vacant
		Chelavoor	Peruvannamuzhi	Appangala	
Scientific	1	-	1	-	-
Technical	9	2	4	-	3
Administration	2	1	1	-	-
Supporting	2	-	2	-	-
Total	14	3	8	-	3

and Mahima and five high curcumin and high yielding turmeric varieties, Suvarna, Sudarsana, Suguna, Prabha and Prathibha were released. Two high quality cinnamon varieties, Navashree and Nithyashree and a nutmeg variety, Viswashree were also released.

Protocols for micropropagation of several spice crops and improved vegetative propagation methods were standardized in black pepper, cardamom, clove, nutmeg, cinnamon, cassia and allspice for rapid clonal multiplication of spices. The optimum spacing, nutrient and water requirements for black pepper, cardamom, ginger and turmeric were standardized for different soils. Mixed cropping systems were developed in black pepper and cardamom for increasing the productivity from unit area of land. Organic farming systems were developed in ginger and turmeric. High production technologies were developed in black pepper and cardamom, which resulted in substantial increase in yield.

Eco-friendly integrated strategies involving cultural methods, biocontrol agents, plant products and resistant varieties were developed for the management of pests and diseases of spice crops that resulted in substantial increase in yields and pesticide-

free produce. Integrated management schedules for *Phytophthora* foot rot disease was well adopted by farmers resulting in significant increase in production of black pepper. A Repository of Biocontrol Agents of bacteria, fungi and nematodes affecting spice crops was established to conserve, characterize and document the variability and potential of biocontrol agents. Large scale multiplication of biocontrol agents for distribution to farmers for management of diseases was also under taken.

Post harvest technologies for processing of black pepper, cardamom, ginger, turmeric, nutmeg, mace, clove, cinnamon and cassia were developed. Technologies for preparation of value added products such as salted ginger and white pepper were also standardized.

The improved varieties and technologies developed on propagation, cropping systems, nutrient and water requirement, pest and disease management and post harvest processing techniques were disseminated to farmers and other agencies through publications, training programmes and demonstrations. Large scale multiplication and distribution of elite planting material was also under taken.



# Research Achievements

## I. Mega Project: Collection, conservation, characterization and cataloguing of germplasm of spice crops for yield and other economically important characters

(Project leader: P. A. Mathew)

### 1. Collection, conservation, cataloguing and evaluation of black pepper germplasm (K. V. Saji, Johnson K. George and R. Ramakrishnan Nair)

#### Collection and conservation

Surveys were conducted in Kottiyoor and Kannavam forests (Kannur, Kerala) and Chettalli, Gonicopal and Makuta forests (Coorg District, Karnataka) and 51 *Piper* accessions were collected, which include 9 local cultivars and 42 wild *Piper*. Good variability of *P. nigrum* (wild) was observed at Kottiyoor forest. A few vines with profuse spiking and good setting were collected.

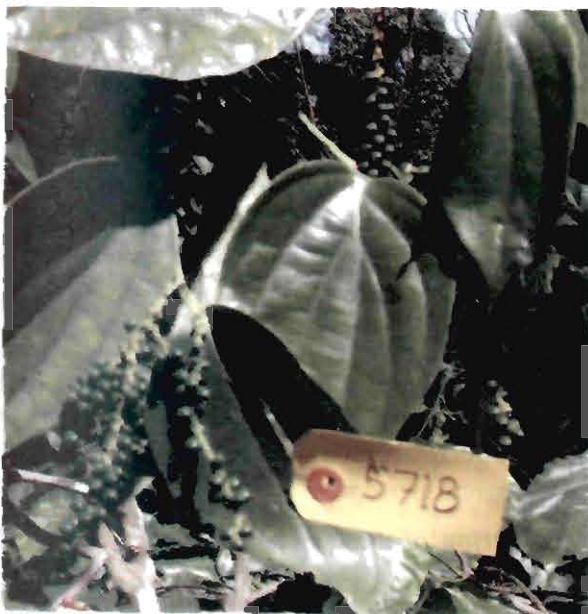


Fig. 2. A black pepper collection with multi-branched spikes from Gonicopal

'Chettalli local'-a local cultivar with very long spike (range: 17-23 cm) and another cultivar with multi-branched spikes (Fig. 2.) were some of the unique accessions collected. An exotic species which closely resembles *Piper colubrinum*, but with erect spikes was collected from Gujarat Agricultural University, Anand (Gujarat).

#### Cataloguing

One hundred accessions of black pepper cultivars were evaluated based on IPGRI descriptor. Among the traits evaluated, yield/vine ranged from 0.015 kg to 2.841 kg with a mean of 0.6 kg. Other characters evaluated were number of fruits/spike, number of berries/10 spikes, weight of 100 fruits, volume of 100 fruits etc., besides morphological and reproductive characters. Quality characters like essential oil, oleoresin and piperine were also evaluated and they ranged from 1.30% to 8.77%, 5.74% to 17.04% and 0.47% to 3.95% respectively.

#### Registration of germplasm

Black pepper accession Coll. 1041, a clonal selection of Thevanmundi was registered with NBPGR, New Delhi (Reg. No. INGR No. 03091), as a unique germplasm having tolerance to foot rot disease.

### 2. Collection, conservation, cataloguing and evaluation of cardamom germplasm

(M. N. Venugopal and K.V. Saji)

#### Collection and conservation

Three accessions of *Alpinium subulatum* and one accession of *Alpinia* sp. were collected

from Sikkim. Three hundred and ninety one accessions of cardamom and their allied genera are maintained in the field repository at Cardamom Research Centre, Appangala.

### Characterization

Forty nine accessions of cardamom were characterized and catalogued based on IPGRI descriptor. Incidence of leaf blight and rhizome rot was recorded in 114 accessions of cardamom and 1 highly resistant, 14 resistant, 54 tolerant, 35 susceptible and 10 highly susceptible accessions were identified. Accessions with erect, semi erect panicles and glabrous leaf characters were resistant to leaf blight and those with prostrate and compound panicles were susceptible to rhizome rot.

Seventy seven accessions of cardamom were evaluated for quality. APG-246, 248, 352 and 378 had more than 8% oil. APG-357 and 365 contained 42%  $\alpha$ -terpinyl acetate with relatively low cineole.

### 3. Collection, conservation, cataloguing and evaluation of germplasm of ginger and turmeric

(B. Sasikumar, K. V. Saji and R. Ramakrishnan Nair)

#### Conservation

Eight hundred and ninety nine *Curcuma* and 659 *Zingiber* accessions are maintained in the germplasm conservatory.

#### Cataloguing

##### Cytological studies in turmeric

Seedling progenies of turmeric were raised from open pollinated seeds of 23 germplasm accessions of turmeric to build up basic material for cytogenetic studies.

Cytological analysis of six seedling progenies from germplasm accessions of turmeric showed normal chromosome number of  $2n=63$  in two accessions (Accs. 730 and 767) and variation in four of the accessions (Acc.

718 =  $2n=68$ ; Acc. 724 =  $2n=78$ ; Acc. 770 =  $2n=72$ ; Acc. 782 =  $2n=72$ ).

#### New varieties of turmeric

Two turmeric varieties namely, IISR Kedaram (Acc. 126) and IISR Alleppey Supreme (Acc. 585). Fig. 3 were proposed for release by the institute after its approval by the XVII All India Coordinated Research Project on Spices Workshop held at Kozhikode. These varieties are high yielding, rich in curcumin and tolerant to leaf blotch disease. Table 5 gives the details of the varieties proposed for release.



Fig. 3. IISR Alleppey Supreme, a turmeric variety proposed for release

#### Stability analysis for rhizome yield

The stability for rhizome yield in 11 new genotypes of turmeric was studied. Pooled analysis of variance for rhizome yield revealed significant differences among the genotypes as well as the environment (Table 6). Stability parameters (mean,  $b_i$  and  $S^2_{d_i}$ ) for the 11 genotypes (Table 7). indicate Accs. 126, 361, 584, 585 and 657 are relatively more stable over seasons as they had nearly uniform values for ' $b_i$ ' and good mean yield.

Acc. 657, a selection of Alleppey Finger Turmeric (AFT) has been registered as a unique germplasm owing to its high yield and high curcumin content.

#### Production of nucleus seeds

The nucleus seed of the varieties released, varieties proposed for release and other *Curcuma* spp. were multiplied. Nucleus seeds

Table 5. Overall mean performance of new turmeric lines.

Variety / line	Dry yield (t/ha)				Pooled mean (t/ha)	Curcumin (%)			Pooled mean (%)	Curcumin (kg/ha)
	Peruvannam uzhi, Kerala	Pundi-baru, West Bengal	Sangli, Maharashtra	Coastal Karnataka		Peruvannamuzhi	Sangli	Coastal Karnataka		
Acc.126	6.6	5.8	3.5	5.3	5.3	5.5	5.6	5.6	5.7	301.1
Acc. 585	7.1	3.9	2.4	9.0	5.6	6.0	4.1	6.0	5.6	309.7
Prabha (Control)	6.0	5.5	3.6	5.2	5.0	6.5	4.9	6.2	5.9	292.5
Prathibha (Control)	6.5	4.9	3.9	5.7	5.2	5.6	5.3	6.3	5.7	300.0

Table 6. Pooled analysis of variance for fresh rhizome yield in turmeric

Source	Df	MSS
Total	43	24.03
Genotypes (G)	10	9.42**
Environment (E)	3	32.63**
G X E	30	29.38**
Environment + (G X E)	33	28.46
Environment (Linear)	1	718.99**
G X E (Linear)	10	15.66**
Pooled deviation	22	2.89**
Pooled error	132	1.79

\*\*P=0.01

Table 7. Stability parameters for rhizome yield in turmeric

Genotype	Mean fresh yield/ 3 m <sup>2</sup> bed (kg)	bi	S <sup>2</sup> d <sub>i</sub>
Acc. 126	12.50	1.19	-5.98
Acc. 295	13.25	0.82	-5.39
Acc. 360	12.25	1.23	-6.67
Acc. 361	13.00	0.96	-5.05
Acc. 584	12.20	0.95	-1.77
Acc. 585	11.38	1.19	-5.81
Acc. 591	9.00	1.71	-4.58
Acc. 593	11.38	1.24	-7.15**
Acc. 656	17.13	-0.18	-0.010
Acc. 657	14.88	1.31	-1.02
Acc. 691	13.50	0.59	-3.46

\*\*P=0.01

of ginger varieties namely, Varada (570 kg), Mahima (748 kg) and Rejatha (618 kg) and turmeric varieties namely, Prabha (538 kg),

Prathibha (800 kg), IISR Alleppey Supreme (250 kg), IISR Kedaram (220 kg), Suguna (37 kg), Sudarsana (33 kg), Alleppey (30 kg) and *Curcuma* species namely, *C. aromatica* (80 kg), *C. caesia* (52 kg) and *C. zeodaria* (65 kg) were produced and distributed to farmers. A portion of the nucleus seed of these lines were supplied for foundation seed multiplication to individual farmers/State Departments against payment.

#### Multiplication of elite ginger accessions

Seven accessions of ginger (Accs. 87, 91, 164, 239, 272, 278, 282) selected from the germplasm for low fibre (2 percent and less) were multiplied. The yield varied from 3.5 Kg to 5.25 kg per 3 m<sup>2</sup> bed.

Sixteen ginger accessions (Accs. 50, 57, 95, 99, 162, 197, 209, 217, 225, 228, 411, 420 and Kakkakalan, Kozhikkalan, Sabarimala, Ellakallan) selected for high oil (2.5–3.0%) were multiplied for evaluation of yield. Fresh yield/3 m<sup>2</sup> bed varied from 3.0 kg to 6.0 kg.

Twelve bold rhizome ginger accessions (Acc. 552, 553, 573, 574, 578, 581, 589, 591, 592, 593, 597, 598) from Nepal were multiplied for yield evaluation. Initial evaluation in non replicated trials revealed that yield/3 m<sup>2</sup> bed varied from 7 kg to 10 kg.

#### Pollination studies in ginger

Sixty flowers were cross pollinated in 13 random cross combinations. No seed set was observed in any of the cross combinations.

#### 4. Collection, conservation, cataloguing and evaluation of germplasm of tree spices

(B. Krishnamoorthy, J. Rema, P. A. Mathew and M. N. Venugopal)

##### Collection and conservation

Eleven collections of kokum (*Garcinia indica*) were collected from Vittal (1), Moodabidri (1), Puttur (4), Puhuvethu (2) and Sediapur (3) and one collection of *G. gummi-gutta* was collected from Chembanode. Four hundred and eight accessions of *Cinnamomum* spp., 482 of *Myristica* spp, 233 of *Syzygium* spp. and 61 *Garcinia* spp. and 180 allspice trees are maintained in the field conservatories and nurseries (Fig. 4). Grafts of 35 accessions of *G. gummi-gutta* were field established for evaluation. One each of *C. glaucens*, *C. camphora*, Konkan Tej, Zanzibar clove graft, 1 high yielding allspice graft (Wyanad) and two *C. perotettii* were field planted.



Fig. 4. A view of the nutmeg conservatory at IISR

#### Cataloguing and evaluation

##### Cinnamon and Cassia

IC numbers for cinnamon and cassia have been obtained from NBPGR, New Delhi.

The performance of elite cassia lines at Peruvannamuzhi in the fifth year indicates no significant difference among the lines for yield.

The elite lines also showed no significant difference, among them for yield attributing characters like height, number of main shoots, no. of branches, canopy width and shoot thickness at fifth year after planting (Table 8). In the evaluation trial at Appangala, significant difference was observed only for height (Table 9). Elite line B-3 recorded the maximum height of 215 cm (2 year after planting). There was no significant difference among the lines for no. of branches.

Planting materials of cassia lines C-1, D-1, D-3 and D-5 were given to KKV, Dapoli; HRS, Pechiparai; RARS, Ambalavayal, and RARS, Sirsi. These trials had been laid out at these 4 centres during July 2003.

RAPD polymorphism was used to estimate the genetic variability in cassia. Genomic DNA was isolated from young fresh leaves of 17 accessions of *C. cassia* using the CTAB method. Four operon primers namely, OPC-11, OPC-12, OPD-06 and OPF-05 were used to develop RAPD profile. All the four primers

Table 8. Performance of clonal progenies of elite lines of cassia (Peruvannamuzhi)\*

Elite Line	No. of harvestable shoots	Fresh weight of bark (g)	Dry weight of bark (g)	Height (m)	No. of main shoots	No. of branches	Canopy width (inches)	Shoot thickness (cm)
A-2	2.4	154.50	27.80	1.92	2.07	13.33	14.63	2.02
C-1	3.2	175.65	41.60	2.35	2.30	15.63	14.46	2.42
D-1	3.6	123.50	28.87	1.96	2.03	13.87	21.08	2.22
D-3	2.2	79.60	21.50	1.95	2.10	11.30	16.96	1.90
CV	44.7	66.58	69.28	-	34.66	48.66	68.18	33.33
CD (P<0.05)	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non significant

\* Fifth year after planting



## 5. Collection, conservation and improvement of vanilla

(R. Ramakrishnan Nair and K. V. Saji)

### Collection and conservation

A total of 30 collections were newly added to vanilla germplasm of these, 29 cultivars were collected from different farmers' field at Wyanad, Kodencherry, Idukki, Pullurampara and Pallimon (Kerala) and South Kanara and Sirsi (Karnataka). The collections made from Sirsi include one with multi-branched inflorescence. One leafless species of vanilla was collected from East Godavari (Andhra Pradesh) by the Project Co-coordinator (spices).

Seventy three collections of vanilla and related species are maintained in the field/nursery at present which include *Vanilla planifolia* (59); *V. andamanica* (8); *V. pilifera* (1); *V. aphylla* (1); *V. tahitensis* (1); *V. vatsalae* (1) and *Vanilla* sp. (leaf less-1).

### Characterization

Morphological characters such as leaf length, leaf breadth, internode length and stem girth



Fig .5. A variant of *Vanilla andamanica*

were recorded from 8 collections of *V. andamanica*. Analysis of variance and comparison of means indicated that Acc. 4704 B is significantly different from other collections with respect to these characters. Floral morphology was recorded in five collections of *V. andamanica*. Acc. 4704 B showed purely white floral parts including the labellum (Fig. 5) while Acc. 4705, 4706, 4709 and 4710 showed creamy white flowers with purple labellum.

### Crop improvement

Pruning of *V. andamanica* during October resulted in profuse flowering in one collection (Acc. 4704 B) and induced flowering for the first time in four others (Acc. 4705, 4706, 4709 and 4710). Flowering was initiated during December to January in four accessions (Accs. 4704 B, 4706, 4709 and 4710) and during March in one accession (Acc. 4705).

Inter-varietal crosses and selfing were done in white and purple flowered varieties of *V. andamanica* and successful fruit set were observed in all combinations. Inter-specific crosses were made between *V. andamanica* and *V. aphylla* and successful seed germination was achieved. About 50 seed derived proto-corms are in different stages of growth.

Seeds resulted from self pollination of *V. planifolia* was germinated *in vitro* in different culture media (SH and E1). Media containing BAP promoted germination of seeds within 40 days. Germination in growth regulator-free media resulted in more normal proto-corms and easy root formation compared to BAP containing medium. Seeds derived from fruits of 4 and 5 months after pollination gave better germination compared to 3 and 6 months after pollination. Initial germination in solid medium and subsequent transfer to liquid suspension resulted in better growth and root formation. About 500 proto-corms at different stages of growth are being maintained *in vitro*. Forty-five seedling progenies were established *ex vitro*.

## 6. Development of paprika for warm humid tropics

(K. N. Shiva and N. K. Leela)

### Collection

Seven accessions of paprika and paprika like chillies (4 exotic and 3 indigenous) were collected from paprika growing areas (IIHR, Bangalore and Coorg District) and added to the existing germplasm, thus raising the total to 47 accessions.

### Purification of germplasm

The newly collected germplasm were raised in the nursery and transplanted to polybags/pots and subsequently purified in insect-proof nylon-net case and the seeds were collected from each accession separately and stored in butter-paper bags under refrigeration at 16 °C.

### Evaluation

Out of the total 40 accessions of paprika, 30 accessions were evaluated for morphological and yield characters (Fig. 6). Among the various characters, wide range (123–746 g/plant) was observed for yield per plant with a mean value of 416 g/plant, while narrow range (0.30–1.03 g/fruit) was recorded for weight of seeds per fruit with mean value of 0.69 g/fruit. Wide variation (10.07%) was recorded for number of seeds per fruit, whereas, narrow variation (1.50%) was recorded for fruit length. Among the



Fig. 6. A high yielding collection of paprika from the germplasm

germplasm of paprika, PBC-171 and Papriking were found to be promising for four characters each, while EC-18, Kt-PI-19, SSP-1999 and ICBD-13 for three characters each (Table 14).

### Quality analysis

Colour value of paprika accessions ranged from 39 to 278 ASTA units with mean value of 184 ASTA units and with variation of 2.46%. The promising accessions were Cayenee, Kt-PI-19, EC-18, EC-71 and ICBD-18 (Table 14).

### Database resources

Database for various morphological yield and quality characters/parameters for the evaluated germplasm (30 accessions) were created and maintained in the computer system.

### Externally Funded Project

#### 1. NATP: Collection, characterization and conservation of spices genetic resources

(Johnson K. George, K. V. Saji, B. Sasikumar and B. Krishnamoorthy)

#### Exploration and collection

Explorations were undertaken in Kottiyoor forest range (Kerala), Makuta forest range and Chettalli in Coorg District (Karnataka). Sixty accessions collected from these areas include wild and cultivated species of *Piper*, *Cinnamomum*, *Garcinia* *Curcuma* (Fig. 7) and *Zingiber*. Population of *P. attenuatum* and *P. nigrum* was found to be high in Kottiyoor forest range. A wide variability for spike length, fruit size, fruit setting and pungency etc. was observed.

Few of the unique collections made during the survey include wild *P. nigrum* with very high pungency, from Kottiyoor forest range; *P. nigrum* (cultivar) from Chettalli with spike length up to 26 cm and good fruit setting; cultivated *P. nigrum* with multibranched spikes (up to 10 branches) from Mymudi village, Karnataka; wild *P. nigrum* with very

Table 14. Evaluation of paprika genotypes for yield, yield attributing characters and quality

Character	Range	Mean	CV %	SD	SE	Promising lines
Days to 50% flowering	54.33-76.33	62.68	2.84	7.56	1.03	PBC-171, Cayennee, EC-43, IMI-5, Papriking
Plant height (cm)	80.00-183.00	123.69	6.05	18.72	4.32	PBC-171, EC-18, EC-71, ICBD-11, ICBD-16
Fruit length (cm)	3.20-17.33	8.57	1.50	2.86	0.07	SSP-1999, ICBD-3, ICBD-13, ICBD-14, ICBD-17
Fruit girth (cm)	4.37-11.77	6.50	4.64	1.90	0.17	PBC-171, EC-71, Papriking, EC-18, EC-45, ICBD - 20
Weight of pericarp (g)	2.41-29.49	6.15	5.01	4.70	0.18	SSP-1999, Kt-PI-19, PBC-171, Papriking, ICBD-1
No. of seeds/fruit	34.00-111.70	70.85	10.07	17.76	4.12	Cayennee, ICBD-2, ICBD-9, ICBD-11, ICBD-12
Weight of seeds/fruit (g)	0.30-1.03	0.69	5.29	0.18	0.02	ICBD-12, Kt-PI-19, Papriking, ICBD-2, ICBD-13
Yield/plant (g)	123.10- 746.60	416.36	6.51	151.94	15.66	EC-18, Kt-PI-19, ICBD-6, SSP-1999, ICBD-13
Color value (ASTA units)	39.00- 277.70	184.26	2.46	56.78	2.61	Cayennee, Kt-PI-19, EC-18, EC-71, ICBD-8

Fig. 7. A *Curcuma* sp. collected from Achankovil forests in Kerala.

high pungency and heavy bearing from Makutta forest (420 MSL); *P. attenuatum* with thick fruit setting; *P. galeatum* from Makutta forest (918 MSL), Coorg District; wild ginger (*Zingiber* sp.) from Kottiyoor forest and *C. riparium* from Kottiyoor forest.

#### Characterization

Fifty each of black pepper, ginger and turmeric germplasm accessions maintained under field conditions were characterized for various vegetative and yield characters.

Inter-simple sequence repeat (ISSR) primers were tested for assessing the suitability of its use in genetic diversity analysis of spices germplasm. Optimum annealing temperature for different primers varied from 42 to 63 °C depending on the base sequences of the primers. All the ISSR primers tested were successful in amplifying inter-microsatellite regions of small cardamom, large cardamom, different species of *Vanilla* and *Piper*. Band

profiles obtained with ISSR primers were found to be highly polymorphic and reproducible. In addition to being simple and time efficient, ISSR-PCR allowed rapid identification of polymorphisms within spices germplasm.

## 2. DBT: Conservation of spices genetic resources in *in vitro* gene banks

(K. Nirmal Babu)

### Conservation

#### *In vitro* gene bank

This year 10 new black pepper collections, 36 lines of ginger and 27 new accessions of turmeric were established. A total number of 750 accessions of various spices are maintained in *in vitro* gene bank by yearly sub culture.

#### DNA Bank

Genomic DNA was isolated from black pepper (species and cultivars), cardamom, ginger, turmeric, vanilla, cinnamon, nutmeg, clove and garcinia and added to the DNA bank making a total of 450.

#### Cryopreservation

Shoot tips of *P. barberi* encapsulated in 4% sodium alginate were pre-cultured on MS medium supplemented with 0.3 M, 0.5 M and 0.7 M sucrose for 4 days followed by dehydration with PVS-2 solution (100%) at 0 °C for 3 h. After dehydration beads were suspended in PVS-2 solution in a cryotube and plunged directly into liquid nitrogen.

Maximum viability (70%) was observed when the encapsulated shoot tips were

treated with 0.5 M sucrose (Table 15.). The cryopreserved shoot tips were regenerated on regeneration media.

#### Genetic fidelity analysis

RAPD profiling was carried out to check the genetic stability of randomly selected five year-old conserved materials of *V. planifolia* and *V. aphylla* using 5 polymorphic operon primers. The gel profile showed no difference in band pattern indicating genetic stability.

## 3. ICAR: Organization of ginger and turmeric germplasm based on molecular characterization

(B. Sasikumar and T. John Zachariah)

A method for isolating good quality PCR amplifiable DNA from powdered samples of turmeric was standardized (Fig. 8).

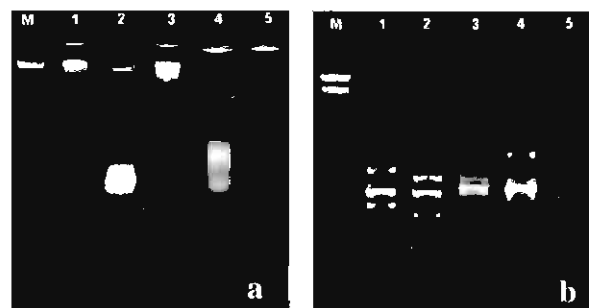


Fig. 8a. DNA samples isolated from turmeric powder. Lane 1-Marker (EcoRI/Hind111 double digest), Lane 2- *Curcuma longa*, Lane 3- *C. zedoaria*, Lane 4 - market sample 1, Lane 5 - market sample 2, Lane 6 - market sample 3. Fig. 8b. RAPD profile of the DNA samples from turmeric powder amplified with primer OPA 2-(5'TGCCGAGCTG 3'). M-Marker (EcoRI/Hind111 double digest), Lane 1- *Curcuma longa*, Lane 2- *C. zedoaria*, Lane 3- market sample 1, Lane 4 - market sample 2, Lane 5 - market sample 3.

Table 15. Effect of various pre-treatments on cryopreservation of *Piper barberi* shoot tips

Treatment	Observation
Pretreatment- WPM + 0.3 M sucrose for 4 days followed by incubation in PVS-2 at 0° C for 3 h.	Buds started germinating after 2 week of post culturing, with a success rate of 40%.
Pretreatment- WPM + 0.5 M sucrose for 4 days followed by incubation in PVS-2 at 0°C for 3 h.	Buds started germinating after 1 week of post culturing with a success rate of 70%.
Pretreatment-WPM + 0.7M sucrose for 4 days followed by incubation in PVS-2 at 0°C for 3 h.	Explants remained viable on post culturing but failed to regenerate.



RAPD analyses of DNA from 96 accessions of turmeric with fifteen random decamer primers showed good polymorphism among the accessions studied.

RAPD analysis of DNA from ninety six ginger accessions with ten random decamer primers yielded low to moderate polymorphism compared to turmeric.

Five species of *Curcuma* were characterized using 12 primers. The intra species polymorphism in *Curcuma* was high as compared to the interspecies polymorphism.

#### Final Report

1. DBT: A digitized inventory of plant resources. Part II – Other economically important species

(P. N. Ravindran and K. Nirmal Babu)

#### Objectives

To build a database on, biological details, national status, utility profiles and economic potentiality of spices and condiments.

#### Documentation and compilation

As part of the nation wide effort in compiling database of plant resources funded by DBT, information was compiled and documented on spices and condiments. The major aspects where information was compiled were taxonomic details (valid name, classification, synonyms, common names), biological details, (habit and habitat, diagnostic features, reproductive biology, breeding system, major

diseases, pests), economic potential, uses etc.

Documentation and compilation of information was completed in 58 spices listed below.

#### Major spices

Black pepper, ginger, cardamom, turmeric, large cardamom, vanilla, long pepper, chilli, onion, garlic.

#### Tree spices

Nutmeg, cinnamon, cassia, bay leaf, clove, curry leaf, carambola, garcinia, kokum, tamarind, star anise and pomegranate.

#### Minor spices

Aniseed, star anise, angelica, caper, greater galangal, horseradish, hyssop, basil, rosemary, parsley, saffron, sage, sweet flag and tarragon.

#### Seed spices

Caraway, black cumin, ajowan, coriander, cumin, fennel, fenugreek, dill, celery, black caraway, black mustard and poppy.

#### Herbal spices

Chives, garden mint, lesser galangal, lovage, peppermint, savory, sweet marjoram, thyme, pandanus, lavender, juniper and oregano.

This data is being compiled at Jawaharlal Centre of Advanced Research, University of Agricultural Sciences, GKVK, Bangalore under the supervision of Professor K. N. Ganeshaiah.

## II. Mega Project: Breeding improved varieties of spice crops for yield, quality, drought and resistance to pests and diseases

(Project Leader: B. Krishnamoorthy)

### 1. Breeding black pepper for high yield, quality, drought and resistance to pests

(B. Sasikumar and Johnson K. George)

#### New varieties

Three new black pepper varieties namely, IISR Thevam; IISR Girimunda (Fig. 9) and IISR Malabar Excel were proposed for release to the State Variety Release Committee after its approval in the XVII AICRP (S) Workshop. IISR Thevam, a



Fig. 9. IISR- Girimunda, a black pepper variety proposed for release

clonal selection of Thevanmundi is a high yielding (5.17 kg/vine), foot rot disease tolerant variety suited to high altitude areas and planes. The hybrid IISR Girimunda (HP-813) is a high yielding clone with 6.14 kg fresh berries/vine suited to elevations of 3000 ft (MSL). The hybrid IISR Malabar Excel (HP-105) though moderate in yield (2.78 kg per vine) is rich in oleoresin (>12%).

#### Yield trials in black pepper

From the yield evaluation trials, laid out at Valparai, HP-728 a hybrid, has been identified as early maturing type with good yield (non replicated trial). This line comes to harvest in the second week of December in planes and by December end in high altitude areas (3000 ft). Table 16. gives the salient features of HP-728 vis-a-vis Panniyur-1, Coll. 1041 and HP-813.

Evaluation of 9 promising lines against Sreekara (control) at Peruvannamuzhi indicated superiority of OPKm, HP-780, HP-1411 and Coll.1041 over the control for yield per vine (Table 17). The performance of few of these promising lines evaluated in farmers' plots in four northern districts of Kerala through the project on Technology Mission on Black pepper also revealed the superiority of OPKm at all locations against the released varieties (Table 18).

Table 16. Evaluation of HP-728 at Valparai, Tamil Nadu\*

Line/ Control	No. of plants	No. of plants yielded			Average yield/plant (kg, fresh)			Mean yield/ vine (kg, fresh)	Dry recov- ery (%)	Esse- ntial oil (%)	Oleo- resin (%)	Piper- ine (%)
		1999- 2000	2000- 01	2001- 02	1999- 00	2000- 01	2001- 02					
Coll.1041**	55	26	38	42	0.15	1.95	2.79	1.63	35	3.2	8.5	1.4
HP-728	54	34	54	48	0.07	2.35	4.00	2.14	33	2.5	8.3	2.9
HP-813**	53	34	51	52	0.54	2.97	3.37	2.29	33	2.8	11.7	2.4
Panniyur - 1*** (Control)	19	-	15	9	-	0.40	2.67	1.53	30	2.0	9.4	3.0

\* Year of planting 1997; \*\*proposed new lines; \*\*\* released hybrid

**Table 17.** Performance of black pepper lines at Peruvannamuzhi

Line/ Variety	Fresh yield/ vine (kg)	Dry recovery (%)	Remarks
OPKm	5.07	31.5	Early variety
HP-1	3.31	31.0	-
HP-2	1.74	32.0	-
HP-780	4.66	38.0	-
HP-1411	4.29	30.5	-
Coll. 1041	4.72	32.0	-
Coll. 1365	3.18	29.0	-
Coll. 4133	3.71	32.5	-
Coll. 8891	3.04	31.2	-
Sreekara	3.97	32.0	-
CD (P=0.05)	1.05	-	-
CV%	16.20	-	-

Seventeen clonal lines and seven OP lines of Neelamundi were evaluated for yield. The yield of clonal lines varied from 0.305 kg (Acc. 878) to 2.0 kg per vine (Acc. 1061 and Acc. 1441). As compared to clonal lines OP lines varied widely for yield per plant.

#### Intervarietal hybridization

In order to converge the genes for resistance

to foot rot, pollu beetle, nematode and drought (multiple resistance) 52 intervarietal crosses between the resistant source lines/ varieties were attempted and seeds were collected and sown.

#### RAPD profiling of different nodal explants

Different nodal explants of two black pepper clones (Panniyur-1 and Sreekara) derived from bamboo method was examined using RAPD profiles to study whether intraclonal variability of rooted cuttings existed. DNA extracted from top, middle and bottom nodal rooted cuttings of the two clones was amplified with 10 random decamer primers. The primers generated 120 amplified products in Panniyur-1 and 124 products in Sreekara. Number of amplified products ranged from 2 to 8 in case of Panniyur-1 and 2 to 6 in case of Sreekara. The ten random decamer primers did not produce any polymorphic bands in the different nodal explants of the two clones barring a single band produced by one primer in the bottom nodal explants of Sreekara.

**Table 18.** Mean yield of promising black pepper lines cultivated in farmer's plots in different northern districts of Kerala

Line/ hybrid/ variety	Kannur	Kozhikode		Wyanad	Kasaragod
	Mean fresh yield (kg/vine)	Mean fresh yield (kg/vine)	Dry recovery (%)	Mean fresh yield (kg/vine)	Mean fresh yield (kg/vine)
OPKm	7.70	4.77	32.8	0.53	1.23
Coll.1041	-	3.14	29.55	0.47	0.25
HP-34	1.78	1.9	30.8	-	-
HP-105	-	2.17	32.75	0.41	-
HP-728	-	2.66	28.9	-	-
HP-780	-	1.9	34.1	-	0.25
HP-1411	-	1.7	24.0	0.24	-
Panniyur-1	2.12	2.2	28.6	-	0.13
Panniyur-2	3.06	4.34	30.17	-	0.08
Panniyur-3	3.36	4.27	31.25	0.6	0.22
Panniyur-4	5.26	5.52	29.6	-	0.13
Panniyur-5	6.34	2.18	30.9	-	0.18
Sreekara	-	1.5	31.2	0.62	-
Panchami	0.89	-	-	-	-

## 2. Biotechnological approaches for crop improvement in black pepper

(K. Nirmal Babu and M. Anandaraaj)

### Molecular characterization of mapping population

RAPD profiles were developed with random primers in 96 progenies of Panniyur 1 x Karimunda cross and scoring of these RAPD loci is in progress (Fig. 10). The total number of RAPD primers used so far are 40. About 160 loci were scored.

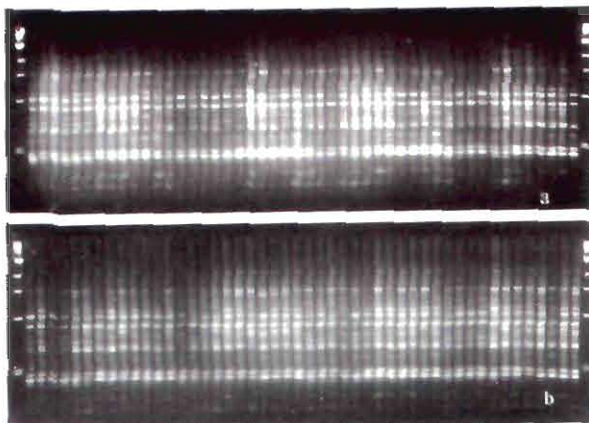


Fig. 10. RAPD polymorphism in mapping population of Panniyur 1 x Karimunda 1 kb ladder, MP-1,2,4,8,11,12,13,15,16,17,20,21,22,24,25,26,28,29,31,34,37,38,43,44,45,46,49,50,51,53,56,57,58,59,60,62,63,65,70,71,72,77,79,80,81,82,83,84,1, kbladder,1kbladder,85,86,87,88,91,94,97,99,100,101,104,105,106,09,110,112,113,116,117,118,123,124,127,129,136,141,143,146,149,154,159,161,162,164,165,170,171,176,179,183,184,185,187,221,255,260, P-1,K-1, 1 kb ladder

### Screening of mapping population for disease resistance

Total number of 169 hybrid progenies of Panniyur-1 and Subhakara were screened for studying the segregation of the parental characters both by leaf as well as stem inoculation methods.

#### Leaf inoculation

Both Panniyur-1 and Subhakara were susceptible to *Phytophthora* infection and had a leaf lesion of 24.3 mm and 45.3 mm respectively. In their crossed progenies the leaf lesion ranged from <5 mm to >20 mm.

Among the hybrids, three (1.78%) progenies were indexed 0, 13 progenies (7.70%) were indexed 1, 57 progenies (33.73%) were indexed 2 and 96 progenies (56.80%) were indexed 3, where score 0 is resistant and 3 most susceptible.

#### Stem inoculation

Panniyur-1 as well as Karimunda is susceptible with a lesion length of 55 mm (the entire internode was infected) and 50 mm respectively. In hybrids the stem external lesion ranged from 0 mm to >20 mm. Twelve (7.10%) of the hybrids were indexed 0, 95 progenies (56.21%) were indexed 1 and 62 progenies (36.69%) 2. Among the hybrids 12 progenies showed resistance to *P. capsici* and 95 progenies showed some degree of tolerance.

#### Depth of penetration index

Data on the depth of penetration of infection was also recorded in the parents and their hybrid progenies as an index for their disease resistance. They were grouped under the following groups 0-4 based on the depth of penetration expressed as percentage on the total diameter of the stem.

Stem penetration index of both the parents Panniyur-1 and Karimunda was 4 i.e. 100% of the stem was internally infected. In the hybrid progenies the index ranged from 0 to 4 of which 12 progenies (7.1%) were indexed 0. In case of hybrids 12 (7.1%) progenies were indexed 0, 44 progenies (26.04%) were indexed 1, 77 progenies (45.56%) were indexed 2, 17 progenies (10.06%) were indexed 3 and 19 progenies (11.24%) were indexed 4. The study showed that there are differences in the parents and the progenies with regard to leaf and stem screening and these characters segregated independently indicating that disease resistance is controlled by more than one gene. The degree of resistance also showed a sort of continuous variation indicating either quantitative or multiallelic interaction controlling disease resistance. Though susceptible Panniyur and Karimunda throws off resistant lines in the segregating populations indicating that they

can be used as parents in hybridization programme to produce resistant hybrids, studies are in progress to further strengthen these findings.

### 3. Development of DNA markers for marker assisted selection in black pepper

(Johnson K. George and B. Sasikumar)

Three ISSR primers (non-anchored) were tested for assessing the suitability of its use in genetic diversity analysis of spices germplasm. DNA was extracted from fresh leaf samples of *Piper* species using cetyltrimethylammonium bromide method or its modifications. Amplified products were electrophoresed on 2% agarose gels. After staining with ethidium bromide, the banding patterns were detected under UV light. Optimum annealing temperature for different primers varied from 42 to 65 °C depending on the base sequences. Band profiles obtained with ISSR primers were found to be highly reproducible. The selected ISSR primers were useful in amplifying inter-microsatellite regions of different species of *Piper* and for identifying selected cultivars of black pepper. ISSR-2 and ISSR-3 primers could distinguish Karimunda, Neelamundi and P-24 from Aimpiriyam and HP-780 which showed identical banding pattern with ISSR-2. This approach also facilitated identification of hybrids in black pepper. ISSR-2 was successful in identifying true hybrids of HP-780 x *P. nigrum* (wild) from the hybrid populations. The hybrid was identified based on the male parent specific band present in the hybrid and the male parent. ISSR-PCR was also found to be successful in differentiating the released varieties of black pepper.

RNA was isolated from the leaves of black pepper (var. Sreekara) having spikes with developing berries and juvenile plants for identification of genes involved in quality attributes. DDRT-PCR was carried out using the oligo dT primer for the first strand synthesis followed by second strand synthesis using four arbitrary 13 mer primers. The cDNA fragments were resolved on 2% agarose gels stained with ethidium bromide.

Up regulation and down regulation of genes were observed while comparing the cDNAs of the two samples. The up regulated genes corresponding to the variety having spikes were eluted for further cloning and sequencing experiments.

### 4. Breeding cardamom for high yield and resistance to 'katte' disease

(M. N. Venugopal)

#### New CYT trials

Two new CYT (NCYT-3 and 4) were laid out with 14 and 13 elite selections and hybrids. NCYT-3 comprises Malabar types and 5 hybrids. Six compound panicle types, 2 semi erect panicle selections, 2 hybrids and 3 land races (Green Gold, Palakuzhy and Wonder cardamom) are included in NCYT-4.

#### Hybridization

Crossing was carried out in 19 combinations in cardamom using 6 parents selected for specific characters like yield, quality, drought tolerance, tolerance to rhizome rot and resistance to leaf blight and mosaic disease. The percentage of fruit set in above combinations varied from 22% to 66%. The seedlings from these crosses were raised for next phase of field evaluation.

#### Screening of hybrids against mosaic virus

Thirty one hybrids of mosaic resistant selections, eight OP selections and twelve compound paniced selections are in the process of screening. The clonal entries were subjected to two rounds of screening against local severe *katte* isolate through viruliferous apterate aphid vector (*Pentalonia nigronervosa* f. *caladii*). Twenty three test accessions have expressed mosaic symptoms.

### 5. Rootstock-scion interactions in tree spices

(J. Rema, P. A. Mathew and K. S. Krishnamurthy)

#### Standardization of grafting in nutmeg

Seeds of *Myristica malabarica*, *M. beddomeii*, *M. fragrans*, *M. prainii* and allied genera *Kuema andamanica* were collected from

different parts of Calicut and from the trees grown in the Experimental Farm at Peruvannamuzhi and all the seeds were raised in the nursery for grafting. Soft wood grafting was carried out with two different scions namely A9-4 and A9-69 on *Knema andamanica* (an allied genera of *Myristica* sp., tolerant to drought) and *M. prainii*. Though success was obtained the percentage of success was not commercially viable. Hence attempts were made to graft nutmeg on *K. andamanica* using *M. malabarica* and *M. beddomeii* as interstocks which are compatible with nutmeg. *M. fragrans* would be grafted on to the interstocks after their successful union on to *K. andamanica*. Grafting was carried out this year also on *M. malabarica*, *M. beddomeii* and *M. fragrans* for producing sufficient grafts for field trials.

#### *Standardization of budding in nutmeg*

Experiments were undertaken this year to standardize budding in nutmeg. Budding was carried out on *M. fragrans*, *M. malabarica* and *M. beddomeii* using green and brown buds. Patch budding and chip budding were attempted. About 40% and 30% success was obtained with chip budding on rootstocks of *M. malabarica* and *M. fragrans* respectively.

#### *Field evaluation of grafts for productivity*

The grafts of nutmeg on three different rootstocks namely *M. fragrans*, *M. malabarica* and *M. beddomeii* using two different scions namely, A9-4 and A9-69 were planted for field evaluation at the Experimental Farm Peruvannamuzhi in a Completely Randomized Block Design. Flowering was initiated in the grafts during the third year of planting and few fruits were also observed. The morphological observations namely height, girth and number of branches were recorded on all the field planted grafts and it was observed that growth was highest on *M. beddomeii* rootstocks for both A9-4 and A9-69 scions. This was followed by *M. malabarica* and *M. fragrans* respectively. In general it was observed that the growth of grafts with A9-

69 scions was superior to that of A9-4. A field trial for evaluating the grafts is in progress in farmers plot at Kolhi Hills and Pollachi in Tamil Nadu.

#### *Field evaluation of grafts for drought tolerance in nutmeg*

A new field trial for evaluating the rootstocks for field performance has been laid out this year in a Completely Randomized Block Design at Peruvannamuzhi. Three different rootstocks namely *M. fragrans*, *M. malabarica* and *M. beddomeii* grafted with two different scions namely A9-4 and A9-69 with 10 plants in each replication was planted at a spacing of 4.5 m X 4.5 m. In the existing trial all the grafts have not established in spite of providing artificial shade and irrigation due to lack of natural shade in the plot. Hence jack seedlings were planted this year. Sixty per cent of the shade trees have established.

#### *Evaluation of clove grafts for dwarfness*

Dwarf clove was grafted on to ordinary clove and vice versa to study the effect of rootstock on inducing dwarfness. It was observed that on grafting dwarf clove on ordinary clove the characters like short internode has been reverted. Further physiological studies are needed to confirm the finding.

#### *Multiplication of clonal rootstock of clove*

Standardization of clonal multiplication of *Syzygium heynianum*, a compatible rootstock for clove, through cuttings and approach grafting is in progress. Zanzibar clove was multiplied through approach grafting on its own rootstock with 80% success.

#### **Final Report**

##### **1. Cytogenetics and reproductive biology of major spices**

(R. Ramakrishnan Nair, K. P. M. Dhamayanthi, B. Sasikumar, K. Padmini and K. V. Saji)

##### *Objectives*

- To study natural polyploidy and its

- distribution in black pepper, ginger, turmeric and cardamom.
- To study cytotaxonomic relationship between *Piper nigrum* and related species.
- To induce artificial polyploidy in black pepper and ginger cultivars.
- To conduct detailed study of the reproductive biology of ginger and other major spices.
- Through various pollination mechanisms, achieving seed set in ginger and production of hybrid ginger.

### Black pepper

#### Chromosome number

A total number of 331 accessions including 227 cultivars and 54 wild types of *Piper* were cytologically analyzed for determining the chromosome numbers using the staining technique standardized. Pretreating the root tips with  $\alpha$  - bromonaphthalene at 11.00 am, fixation at 2.00 pm, hydrolysis with 1N HCl and staining with lactopropionic orcein were found to be ideal for black pepper. Accessions Acc. 396 (*Piper attenuatum* (male) -  $2n=8x=104$ ), Acc. 656 (*Piper* sp. -  $2n=8x=104$ ), Acc.1344 (*Piper nigrum* cv. Vadakkan -  $2n=6x=78$ ), Acc. 3329 (*Piper* sp. -  $2n=125, 118, 106, 86,$  ) and Acc. 4509 (*Piper argyrophyllum* -  $2n=7x=91$ ) showed variable chromosome number than normal  $2n=52$ .

Numerical variation was observed among the OP progenies of Col. No. 1344.

Chromosome number of *P. barberi*, an endangered species was reported as  $2n=52$ . The chromosome numbers of *P. arboreum*, *P. colubrinum* and *P. magnificum* (South American species) were confirmed as  $2n=26$ . Female plants of Jawa long pepper (*P. chaba*) have  $2n=104$ . A wild species collected from North-Eastern region was reported to have  $2n=91$ .

#### Karyotype analysis

Karyotypes of black pepper cultivar

Kurialmundi and wild species *P. magnificum* and *P. colubrinum* were prepared using mitotic metaphase plates. Chromosome length ranged from 0.66 to 1.33  $\mu\text{m}$  and genomic formula was found to be  $16m + 9sm + 1st.$  in *P. nigrum* cv. Kurialmundi (Acc. 917). In *P. magnificum* chromosome length ranged from 1.41 to 3.34  $\mu\text{m}$  and genomic formula was  $7m + 6sm$ . Karyotype analysis of *P. colubrinum* revealed that chromosome length ranges from 1.08 to 3.26  $\mu\text{m}$  and have seven pairs of metacentric, five pairs of submetacentric and one pair of acrocentric chromosomes. Chromosome pairs No.1, 2, and 4 were having secondary constrictions.

#### Induction of polyploidy

Experiments to induce polyploidy in black pepper was started even before the commencement of the project. An induced tetraploid (C-5) with  $2n=104$  was identified from a population of plants derived from the colchicine (0.05%) treated seeds of Panniyur-1. The induced polyploid was multiplied vegetatively and planted in the field during 1993-94 for further observation. In general, the cuttings showed stunted growth but a few of them had normal growth. One of these vines produced spikes with medium length, sparse setting and bold berries, but none of the seeds germinated.

#### In vitro androgenesis

Segments of spikes and anthers were cultured on E1 medium supplemented with and without growth regulators such as BAP and NAA. Segments of spikes having anthers with uninucleate pollen when cultured in hormone free E1 medium (Gamborg, 1983) under 24 h. dark period initiated the growth of anthers without callusing. But no organogenesis/embryogenesis occurred from such anthers.

#### Zygotic embryo development

Histological analysis of developing fruits and germinating seeds of black pepper revealed that by three months after pollination the embryo attains 'heart-shaped' structure. This



'heart-shaped' embryo remains in the same stage till seed ripen and further development takes place only after seed shedding, during the process of germination. Studies on zygotic embryo structure in *P. mullesua*, and *P. colubrinum* indicated similarity in structure to *P. nigrum*, except for the small size. Preliminary observations indicated that zygotic embryo is a 'top-shaped' structure in *P. longum*.

## Ginger

### Chromosome number and morphology

Among the forty two accessions analyzed for chromosome number Acc.116, Acc. 147 and Acc. 246 (Sabarimala) have  $2n=24$  and all others have normal number of  $2n=22$ .

Karyotype analysis and *in situ* estimation of 4c nuclear DNA content indicated wide difference between cultivars China and Maran. Karyomorphological analysis showed significant variation in total chromosome length, distribution of secondary constriction and SAT chromosomes, between Acc. 246 and var. Varada.

### Reproductive biology and pollination studies

Studies on pollen germination indicated that the average germination is 29% and average pollen tube length is 272  $\mu\text{m}$ . Abnormal pollen germination was observed in 16% of the pollen. Histological studies showed normal ovule development indicating the possibility of high ovule viability. Heterostyly of pistils was observed showing 'pin' and 'thrum' type styles. Flowers of ginger opens between 2.45 pm and 3.00 pm and anthesis occurs 15 minutes after flower opening. Flowers fall off by next day morning. Self pollination of 309 flowers from 32 accessions of ginger has not resulted in any seed set.

### Polyploidy induction

Treating buds with 2% colchicine induced three polyploids of IISR Varada. Attempts to induce polyploidy in other cultivars such as

Maran, Mananthody and Suprabha was not fruitful even though some plants showed polyploid symptoms at early stages.

## Turmeric

### Reproductive biology

Observations on reproductive biology of turmeric indicated that the time required for the appearance of inflorescence is 102 to 116 days and number of flowers per inflorescence ranged from 47 to 55. The opening of flowers start from 7.00 am and continue up to 4.00 pm. The time of anther dehiscence is between 10.15 and 11.40 am usually.

Pollen fertility studies in 5 accessions of turmeric showed that even though pollen stainability is reasonably high (53 to 58%), actual germination is less than 10% in Brewbacker and Kwak medium (1963) containing 20% sucrose. Germination started within 30 minutes of culture. Pollen tube attain a length of even 2.5 mm *in vitro*.

### Seed and embryo structure

Histological analysis of fruits and seeds of *Curcuma longa* showed that the seeds are attached to a central column inside the fruit. Different seeds derived from the same fruit showed embryos of different developmental stages occasionally. The monocotyledonary embryos resembled that of cardamom in structure. Six different developmental stages of embryos from early globular to cotyledonary stages were identified by histological analysis of seeds from fruits of different developmental stages. Persistence of nucellus was evident in the mature seed.

### Chromosome number

Cytological analysis of OP seedling progenies and germplasm accessions indicated frequent variation from normal chromosome number ( $2n=63$ ) in seedling progenies and occasionally in germplasm accessions also.

### Seed germination

A preliminary study on seed germination of



turmeric using two accessions (Acc. 126 and 399) showed that only few seeds germinated within 1 month of sowing and majority of seeds germinated after 5 months of sowing with 75% and 73% germination in Acc. 126 and 399 respectively.

The seeds also germinated *in vitro* on SH and E1 medium. In these media also only a small percentage of seeds germinated within 1 month. Attempts to germinate seeds in 2, 4-D containing medium produced profuse white callus with high potential of plantlet regeneration in medium containing BAP.

### Cardamom

#### Cytological studies

Chromosome number analysis of 18 accessions of cardamom including 9 multibranched types revealed that all of them are having normal chromosome number of  $2n=48$ . Cytological analysis in four species of *Amomum* namely, *A. subulatum*, *A. muricatum*, *A. microstephanum* and *A. canneicarpum* revealed that all of them are having  $2n=48$ . But, the difference in size of chromosomes among these species was evident.

#### Future line of work

- Meiotic studies in all the above crops.
- Sterility problem and lack of seed set in ginger has to be investigated further.
- Variation in chromosome number in seedling progenies of turmeric should be studied in detail by analyzing the progenies of single parents for a clear understanding of triploid segregation in turmeric.
- Advanced methods of cytological characterization such as measuring nuclear DNA content may be explored in black pepper, as the chromosomes are very small and manual karyotyping is often confusing.

## 2. Large scale multiplication of released varieties of black pepper through somatic embryogenesis and genetic fidelity testing

(R. Ramakrishnan Nair and Johnson K. George)

### Objectives

- Multiplication of selected released varieties of black pepper through somatic embryogenesis.
- Characterization of regenerated plants through morphological, cytological, biochemical and molecular parameters.

### Induction of somatic embryos

Primary somatic embryogenic cultures were established in var. Subhakara by culturing seeds in growth regulator free SH medium under full darkness. The process was scaled up by secondary cyclic embryogenesis by sub-culturing the primary embryos. The plantlets were regenerated in liquid SH medium. Sixty plants were regenerated and established *ex vitro*.

### Cytological analysis

Cytological analysis of 40 regenerated plants indicated normal chromosome number of  $2n=52$ .

### RAPD analysis of regenerated plants

Four random operon primers namely, OPE-2, OPD-3, OPF-5 and OPD-16 were used to develop RAPD profiles of somatic embryo derived plants of black pepper Subhakara, in comparison with the mother plant. Profiles generated by operon primers OPE-2, OPD-3, and OPD-16 produced a uniform pattern among the 22 somatic embryo-derived plants in comparison with the mother plant indicating genetic uniformity. However, the banding patterns developed by operon primer OPF-5 showed some polymorphism in four of the regenerated plants. Analysis of another set of 19 plants using OPE-11 indicated differences in few bands in four of the regenerated plants.

### Future line of work

Regeneration of a large number of plants from somatic embryos of different cultivars and genetic fidelity testing may help to develop an efficient technology for micropropagation of black pepper.

### Externally Funded Project

#### 1. DBT : Improvement of selected spice crops through biotechnological approaches

(K. Nirmal Babu, Johnson K. George, M. Anandaraj, M. N. Venugopal and R. Ramakrishnan Nair)

#### Cardamom

##### Molecular characterization

RAPD profiles were developed using 30 random primers in 11 related genera of cardamom, namely *Elettaria cardamomum*, *Amomum subulatum*, *A. ghaeticum*, *A. microstephanum*, *A. meluguta*, *A. involucreatum*, *Alpinia purpurea*, *A. galanga*, *A. mutica* and *Hedychium coronarium*. All the primers exhibited good polymorphism between the genera. Species inter relationships were studied. Among the genera *H. coronarium* was found to be nearest to cardamom while the farthest was *A. microstephanum* among the genera studied. RAPD profiles were also developed using 50 random primers in 24 important varieties and promising lines namely, ICRI-1 and 2, RR-1, NKE-9, NKE-12, NKE-19, CCS-1, PV-1 and 2, Mudigere-1 and 2, Sampajie clone, MCC lines 12, 21, 40, 85, 346, S-1, PS-27, SKP-165 and 170, MHC-26 and 27 and Hybrid-3.

In view of the limited polymorphism observed in RAPD profiles of cardamom a modified RFLP-PCR was standardized by restricting the PCR products of random primers with restriction enzymes before visualizing the gels. When Bam HI and Eco RI were used the number of bands increased so is the level of polymorphism. This method was found to be better than standard RAPD profiling in the case of cardamom.

Inter Simple Sequence Repeat (ISSR) reaction conditions were also standardized in cardamom. Primers were designed with 2 anchored bases at 3' end for higher specificity. The conditions standardized for *E. cardamom* is 150 mM of dNTP, 2 mM MgCl<sub>2</sub>, 30 ng of genomic DNA, 30 ng of primers, 1U Taq DNA polymerase in a 25 ml reaction volume optimum temperature profiles with initial denaturation at 94 °C for 3 minutes a core cycle of 94 °C for 1minute, 50 °C for 1minute, 72 °C for 1 minute for 30 cycle followed by the final cycle of 72 °C for 10 minute were found to be good.

The data generated by all these 3 markers were used in studying the distance, similarities and inter relationships between the important varieties and collections of cardamom. The studies indicated that all these varieties are different from each other and there are no duplicates in them.

#### Black pepper

##### Genetic transformation

Plantlets were successfully regenerated from black pepper seedling explants treated with *Agrobacterium* strain GV-2260 containing osmotin procured from NRC Plant Biotechnology, New Delhi (Fig. 11). Five plants regenerated from *Agrobacterium* treated leaf explants of black pepper in selection medium containing kanamycin were planted out. Ten more plants were regenerated using hypocotyls explants.



Fig. 11. Plant regeneration from *Agrobacterium* treated tissues of black pepper

##### Confirmation of transgeneicity

PCR amplification was done with osmotin

and npt II specific and a few putative transformants showed 550 bp product specific for the osmotin. A 700 bp PCR product was obtained when primers specific for npt II were used indicating their transgeneity.

#### *Amplification of $\beta$ -1, 3 glucanase genes from *P. colubrinum**

A gene targeted approach similar to that for isolating chitinase gene was used in amplification of  $\beta$ -1, 3 glucanase genes from *P. colubrinum*. A 15 mer primer was designed based on partially conserved amino acids of glucanase genes. The primer was successful in amplifying a 350 bp band which was found to be differentially expressed in the RNA sample of *P. colubrinum* challenged with *P. capsici*.

#### *Differential display amplification*

The RNA isolated from *P. colubrinum* leaves (control and challenged with *P. capsici*) were subjected to reverse transcription using oligo d (T) 15 mer primer for the first strand synthesis followed by random 13 mer primers for the second strand synthesis. Five random primers were successful in differentiating gene expression in the challenged *P. colubrinum* plants from that of the control. The amplification products (cDNAs) corresponding to differentially expressed mRNAs were eluted for further cloning and sequencing.

#### *Cloning of differentially expressed genes*

The differentially expressed bands of putative chitinase,  $\beta$ -1, 3 glucanase and *Phytophthora* resistance genes were eluted from the agarose gel, purified and reamplified using the same primers. The PCR products were run on 1% agarose gel to confirm the presence of the desired bands. The TA cloning kit (Invitrogen) containing the vector pCR 2.1 was used for the direct insertion of the PCR product into the plasmid vector. The insert and vector was ligated using T4 DNA ligase and were transformed into competent DH5 $\alpha$  cells. The transformed colonies were plated on LB agar

plates containing X-gal, IPTG and 50  $\mu$ g/ml of kanamycin. The white colonies were picked up for plasmid isolation and were subjected to restriction digestion using ECoRI to find the presence of insert. The recombinant plasmid was also subjected to polymerase chain reaction using the specific primers to confirm the presence of the clone. The cDNA's corresponding to the 3' end of the resistance genes were sequenced (Avesthagen Technologies). The putative resistance gene was found to have more similarity with a putative resistance gene from *Arabidopsis*.

#### **2. DBT: On-farm evaluation of tissue culture derived plants of black pepper**

(K. Nirmal Babu, M. Anandaraj, V. Srinivasan and R. Ramakrishnan Nair)

#### *Multiplication and field planting*

Six thousand multiplying, *in vitro* cultures of two released varieties of black pepper namely, IISR Subhakara and Panchami were established and are in various stages of multiplication. Two hundred good healthy rooted cultures of black pepper (Subhakara and Panchami 100 cultures each) were handed over to Biotechnology and Model Floriculture Centre Kazhakuttom, for further multiplication.

Six yield trails were planted last year in IISR Experimental Farms and farmer's field. The trials at IISR is with 300 plants, the establishment was over 80%. *Trichoderma harzianum* at the recommended dose was used at the time of planting.

#### *Genetic fidelity analysis*

RAPD and ISSR profiling of 20 randomly selected tissue cultured plant of vars. Subhakara and Panchami, was done using more primers and the profiles indicated genetic uniformity among the micropropagated plants. RAPD and ISSR characterization of 20 randomly selected somatic embryo derived plants gave uniform profiles indicating genetic similarity.

### III. Mega Project: Soil and crop management studies for augmenting spice productivity

(Project Leader: K. Kandiannan)

#### 1. Nutritional requirement of improved varieties of spices

(V. Srinivasan, K. S. Krishnamurthy, C. K. Thankamani and S. Hamza)

##### Evaluation of micronutrients

In black pepper, application of Zn either through soil (@ 5 kg Zn/ha) or as foliar spray (0.25% twice) has yielded on par in each location studied (Table 19). But the pooled analysis of all the three locations revealed that soil application of Zn recorded significantly higher spike intensity and yield than foliar or no Zn application.

The effect of levels of Zn in combination with coir pith compost was studied on ginger and turmeric. The application of graded levels of Zn increased the soil DTPA Zn availability significantly up to 11.17 ppm as compared to control, but the yield was on par among the levels studied as the yield levels were low (Table 20). The levels of coir pith compost applied had significant increase in soil P availability. The optimum fertilizer dose for obtaining maximum rhizome yield was 6 kg ha<sup>-1</sup> of Zn and maximum limit of soil DTPA-Zn for getting higher rhizome yield was found to be 3.4 mg kg<sup>-1</sup> through a cubic model.

In turmeric, the soil DTPA Zn availability increased significantly with the levels of Zn applied and the rhizome yield recorded was

significantly high at 2.5 t coir pith compost application (18.33 t/ha) (Table 21). Among the levels of Zn applied significantly highest rhizome yield of 19.23 t/ha was observed at 7.5 kg Zn/ha application which was on par to other higher levels of soil application of Zn and foliar application (@ 0.25% 2 sprays).

##### Targeted production in black pepper

The mean yield recorded in black pepper vines that are targeted with 10, 15 and 20 kg fresh yield/vine are 13, 13.9 and 16.4 kg/vine respectively (Table 22). The deviation from the fixed targets was +30%, -6.9% and 19.0% respectively, showing that at targets levels of 15 and 20 kg/vine, up to 93 and 81% of fixed yield levels could be achieved. At 10 kg/vine target, the vines out yielded the target.

##### IPNM schedules in ginger and turmeric

The treatments with integrated application of nutrients have shown significantly increased yield in ginger with FYM + ½ N and P + K + phosphorous solubilizing bacteria (PSB) + NC application (21.5 t/ha) over recommended package of practices (18.9 t/ha), whereas the nutrient build up was on par except that of N (Table 23). In turmeric, integrated application of PSB and neem cake (NC) along with chemical sources significantly increased the soil available N and P status and the highest rhizome yield was recorded in FYM + ½ N + PSB + NC + P and K application (23.8 t/ha) (Table 24).

**Table 19.** Effect of zinc application on spike intensity and yield of black pepper

District	No. of spikes 0.25m <sup>2</sup> /canopy				Yield (kg/vine)			
	No Zn	SZn	F Zn	Mean	No Zn	SZn	F Zn	Mean
Kasaragod	9.9	12.0	9.4	10.4	1.1	1.6	1.2	1.29
Kannur	14.6	14.5	8.3	12.5	2.3	2.5	1.8	2.21
Calicut	24.4	19.0	16.6	20.0	2.3	2.6	2.5	2.45
Mean	16.3	15.2	11.4		1.8	2.2	1.8	
CD (P <0.05) Loc - 1.86; Treat 0.38								
Loc x treat 3.23								
								0.66

NoZn : No Zinc, SZn : Soil Zinc; FZn : Foliar Zinc

**Table 20.** Effect of zinc with and without coir compost on P and Zn availability (mg/kg) and yield (kg/3 m<sup>2</sup> bed) of ginger

Zn (kg/ha)	Soil Zn			Soil P			Yield		
	W/o	W	Mean	W/o	W	Mean	W/o	W	Mean
0	5.1	5.2	5.15	47.3	51.0	49.2	5.8	6.4	6.05
5	8.0	7.0	7.47	46.7	44.3	45.5	5.8	6.0	5.92
7.5	8.6	8.7	8.63	39.7	55.0	47.3	5.7	5.6	5.62
10	11.9	8.6	10.25	51.3	54.7	53.0	5.5	5.5	5.51
15	11.8	10.5	11.17	41.7	48.0	44.8	5.3	5.5	5.35
Foliar	6.0	6.6	6.33	33.7	46.3	40.0	5.0	6.7	5.84
Mean	8.6	7.8		43.4	46.9		5.5	5.9	
CD (P<0.05) for Zn levels		1.98			6.46			NS	
CD (P<0.05) for CC		NS			3.73			NS	

W/o = Without coir pith ; W = with coir pith ; CC = Coir compost

**Table 21.** Effect of zinc with and without coir compost on P and Zn availability (mg/kg) and yield (kg/3 m<sup>2</sup> bed) of turmeric

Zn level kg/ha	Soil Zn			Soil P			Yield		
	W/o	W	Mean	W/o	W	Mean	W/o	W	Mean
0	2.5	3.1	2.78	58	53.3	55.7	7.4	7.8	7.6
5	6.5	5.4	5.98	58	48.3	53.2	7.7	9.1	8.4
7.5	8.2	7.4	7.8	44.3	50.3	47.3	8.7	10.5	9.6
10	9.6	7.6	8.6	49.3	48.0	48.7	8.0	10.3	9.2
15	10.5	12.4	11.43	54.3	46.0	50.2	8.6	9.3	8.9
Foliar	3.0	2.5	2.78	53.3	41.7	47.3	7.8	8.1	8.0
Mean	6.7	6.4		52.8	47.9		8.0	9.2	
CD (P<0.05) for Zn levels		1.56			NS			1.8	
CD (P<0.05) for CC		NS			NS			NS	

W/o = Without coir pith ; W = With coir pith

**Table 22.** Investigation on nutrient requirement for targeted production of black pepper

Fertilizer dose (g/vine)			Target yield (kg/vine)	Actual mean yield (kg/vine)	Deviation (%)
N	P	K			
83	30	450	10	13.0	+30.0
160	56	930	15	13.9	-6.9
235	80	1410	20	16.0	-19.0

**Table 23.** Effect of IPNM on soil nutrient availability and yield of ginger

Treatments	N (mg/kg)	P (mg/kg)	K (mg/kg)	Yield (kg/bed)	Yield (t/ha)
FYM+NPK	145.2	43.2	233.0	9.4	18.9
FYM+NC+PB+½N+½P+K	143.0	35.6	230.1	10.8	21.5
FYM+NC+PB+½N+P+K	151.8	37.6	234.4	8.3	16.6
FYM+NC+PB+½N+2P+K	161.4	37.8	230.0	8.7	17.8
CD (P<0.05)	14.4	NS	NS	0.56	1.11

NC= Neem cake ; PB = Phosphorous solubilizing bacteria

**Table 24.** Effect of IPNM on soil nutrient availability and yield of turmeric

Treatments	N (mg/kg)	P (mg/kg)	K (mg/kg)	Yield (kg/bed)	Yield (t/ha)
FYM+NPK	166.0	37.8	235	9.6	19.1
FYM+NC+PB+½N+½P+K	144.1	45.8	247	9.9	19.7
FYM+NC+PB+½N+P+K	153.8	41.8	239	11.9	23.8
FYM+NC+PB+½N+2P+K	167.0	50.4	259	11.8	23.5
CD (P<0.05)	6.08	6.08	NS	1.45	2.94

NC= Neem cake ; PB = Phosphorous solubilizing bacteria

### Organic farming in ginger

Ginger variety Varada was grown organically by applying FYM, vermicompost, ash and rock phosphate and *Pseudomonas sp.* as biocontrol for rhizome rot. The mean yield recorded was 7.3 kg bed<sup>-1</sup> as compared to 5.4 kg bed<sup>-1</sup> in case of conventional farming. In turmeric var. Prathiba has recorded a mean yield of 6.5 kg bed<sup>-1</sup> under organic cultivation as compared to 5.5 kg bed<sup>-1</sup> in conventional system.

### 2. Efficacy of biofertilizers on nutritional management of black pepper

(C. K. Thankamani, K. S. Krishnamurthy, V. Srinivasan and K. Kandiannan)

The field trial to study the influence of *Azospirillum sp.* on growth and yield of black

pepper was continued. Soil samples were collected and analyzed for nutrients before and after imposing treatments. *Azospirillum* population in the soil was counted before and after imposing treatments. Initial nutrient status was high in nitrogen, phosphorous and potash. In the pretreatment *Azospirillum* population was low. The *Azospirillum* population gradually increased and highest counts were recorded for the treatment inorganic nitrogen 50% + Mg. Effect of *Azospirillum* application on NPK content of soil, six months after application of inoculum was not significant. Among the treatments, significantly higher soil nitrogen was recorded in the treatment where FYM application was alone and least soil nitrogen was recorded in the treatment inorganic nitrogen 50% + zinc

sulphate. Maximum amount of phosphorous in the soil was observed for the treatment inorganic nitrogen 50% + 10 kg FYM that was on par with inorganic nitrogen 75% + 10 kg FYM. Significantly higher amount of potash was observed for the treatment inorganic nitrogen 50% + zinc + B + Mo and least in inorganic nitrogen 50% + calcium.

### 3. Biometeorological investigations and modeling in black pepper

(K. Kandiannan, C. K. Thankamani, K. S. Krishnamurthy, V. Srinivasan and Utpala Parthasarathy)

Monthly rainfall and black pepper production of Kerala State for 11 years (1991 to 2001) were collected from Farm Guide published by Farm Information Bureau, Government of Kerala, Thiruvananthapuram. The monthly rainfall (January to December) of current and previous years was correlated with black pepper production. There was no significant relation of previous year's monthly rainfall with current year production whereas, September rainfall of current year alone had a positive significant association ( $r = 0.6094$ ) with pepper production. This sort of correlation and regression studies will help in developing crop-weather model to predict the yield/production in a region based on weather. The black pepper production primarily depends on rain, onset of the southwest monsoon is the commencement of the season and it triggers new flushing and flowering. During this observational year flowering (> 50 %) appeared in the 22<sup>nd</sup> meteorological week (28-5-03 to 03-6-03). The spikes were ready for harvest during 2<sup>nd</sup> meteorological week (08-01-04 to 14-01-04) of subsequent year. The rainfall received during this period was 2823.7 mm. The thermal time required for the maturity was 3676.2 degree days.

### 4. Development and evaluation of soil and water conservation measures and land use system for sustainable crop production in Western Ghats of coastal regions

(S. J. Ankegowda)

#### *Soil and water conservation*

Growth and yield parameters, soil moisture depletion pattern, soil nutrient status, runoff, soil loss and nutrient leached through runoff were calculated in different treatments. Contour staggered trenches were used as soil conservation measures and pine apple, french bean and ginger were used as vegetative barriers. Growth parameters and soil nutrient status was non-significant between treatments.

Soil loss ranged from 124.3 kg/ha to 943.6 kg/ha in different treatments and run off ranged from 3.96 mm to 19.92 mm in different treatments. Soil loss and run off were less in cardamom with contour staggered trenches. Soil loss was low in coffee with contour staggered trenches and ranged from 22.6 kg/ha to 131.5 kg/ha. Runoff ranged from 0.9 mm to 5.53 mm.

#### *Evaluation of planting systems*

Pit system of planting, trench system of planting, scooping and planting, half moon terraces and continuous half moon terracing were evaluated in cardamom for identifying an efficient system for utilization of rain water. Soil loss ranged from 87.82 kg/ha to 524.5 kg/ha. Soil loss was less in trench system of planting and more in half moon terracing for each plant. Run off ranged from 3.5 mm to 22.02 mm and was low in trench system of planting and higher in pit system of planting cardamom.

#### *Assessment of frequency and duration of irrigation in cardamom*

An experiment was conducted in cardamom with four treatments namely a) control-mulching with local material (protective irrigation) b) drip irrigation-8 litre per day c) sprinkler irrigation once in 15 days and

sprinkler irrigation once in 12 days for assessing the frequency and duration of irrigation.

Growth, yield, soil moisture status and soil nutrient status was monitored. No significant variation between treatments for plant height, number of tillers per clump and number of leaves per tiller was observed. Significant variation has been observed for fresh weight of capsule (g/clump) for different treatments. Fresh weight of capsule (g/clump) ranged from 332.37 g/clump to 472.9 g/clump. Fresh weight was highest in sprinkler irrigation once in 10 days (472.94 g/clump) followed by drip irrigation (463.2 g/clump) and lowest in control with protective irrigation once in a month (332.37 g/clump).

#### Externally Funded Project

#### 1. Government of Kerala: Technology mission on black pepper

(V. Srinivasan, B. Sasikumar, C. K. Thankamani and R. Suseela Bhai)

The experiments were conducted in farmers plots spread over 4 northern districts of Kerala state namely, Calicut, Kannur, Wyanad and Kasaragod.

Among the new lines and/or seven released varieties evaluated, OPKm ranked first in Kannur and Kasaragod districts with a mean yield of 7.7 kg and 1.23 kg fresh berries per vine, respectively. It recorded 4.77 kg and 0.6 kg fresh berries per vine at Kozhikode and Wyanad districts respectively. OPKm showed tolerance to drought in all the places.

In the experiment on organic manuring of black pepper, the soil fertility was maintained on par among the treatments studied. At Kasaragod, soil available N and P contents were high in treatments with 50% FYM + N + phosphobacteria. Soil K was significantly high in treatment 50% FYM + neemcake. In Kannur the increase in pH was significant with the addition of organics and neem cake application recorded highest K. Higher

micronutrient Zn availability was observed in biofertilizer applications. The yield recorded was highest in Wyanad and Kasaragod districts followed by Kannur and Calicut districts.

In experiments on standardization of low input technology the P availability of soils in Kannur District was lowest and the K available was significantly highest in ½ POP (recommended package of practice) + Zn application. In Kasaragod, the soil P available was higher with more P in biofertilizer-applied treatments. The micronutrient availability was also high with highest Zn in ½ POP + Zn application. In Calicut, the P availability was highest in POP followed by biofertilizer application and the micronutrient availability was also observed to be high. In Wyanad Ca and Mg availability was also medium with Zn availability just above the critical range. The recorded yield levels among low input treatments were highest in Calicut District followed by Kannur, Kasaragod and Wyanad. Even though the treatments were on par, the highest yield was recorded in treatments with ½ POP + neem cake, ½ POP + biofertilizer, ½ POP + cowpea and ½ POP + Zn applications.

In the experiment on use of biocontrol in checking *Phytophthora* foot rot in the nursery, sprouting was found comparatively higher in treatments where bio-agent was used along with potassium phosphonate. Height of the plants and average no. of leaves/plant was also more with the same treatment in solarized soil. Application of bio-agents twice along with potassium phosphonate or Bordeaux mixture was found to be superior to all other treatments both in the existing plantations and also in the new plantings.

One thousand and eight hundred rooted cuttings were produced from the rapid multiplication nurseries from the four districts. During the year 40,445 rooted cuttings of released varieties namely, Subhakara, Panchami, Pournami, P-1, P-2, P-3, P-4, P-5 and P-24 were produced.



## IV. Mega Project: Production physiology of spice crops

(Project Leader: B. Chempakam)

## 1. Biogenesis of pigments in spice crops

(B. Chempakam and T. John Zachariah)

## Tracer studies

Phenylalanine is the primary precursor in curcumin biosynthesis. Labeled studies using  $1\text{-}^{14}\text{C}$ -phenyl alanine was conducted in one month old turmeric plants through root absorption, to know the rate of conversion of these compounds to phenolic acid, which are the intermediate precursors. The percentage of incorporation of  $^{14}\text{C}$  in the phenolic acid and curcumin in the vegetative parts of turmeric namely, leaf, pseudostem, root and rhizome at different stages of plant growth are given in Tables 25 and 26.

In the leaf, simultaneous incorporation of  $^{14}\text{C}$  in both phenolic acids and curcumin was seen after one month, to the maximum level. In pseudostem, highest incorporation of both the parameters was seen after one week. In rhizomes, lower incorporation was seen in phenolic acids at 48 h, probably due to higher utilization and conversion into curcumin. This is supported by the higher DPM (Disintegrations per minute) observed for curcumin in rhizomes during the same period.

Since malonyl-co-A is also involved in the final stages of biosynthesis, root absorption studies using  $2\text{-}^{14}\text{C}$ -malonyl Co-A were carried out in one month old turmeric plants.

Table 25. Incorporation of  $^{14}\text{C}$  in phenolic acids in the vegetative parts of turmeric during administration of  $1\text{-}^{14}\text{C}$  phenyl alanine

Sampling interval	Plant part			
	Leaf (DPM)	Pseudostem (DPM)	Rhizome (DPM)	Root (DPM)
48 hour	61.3 (1.2)	62.8 (1.3)	36.2 (0.71)	4960.4 (96.9)
1 week	93.9 (15.1)	102.0 (16.4)	243.9 (39.2)	183.7 (29.5)
1 month	105.7 (9.1)	39.6 (3.4)	51.5 (4.41)	969.5 (83.2)
2 month	14.9 (6.8)	28.7 (3.2)	50.5 (23.3)	122.8 (56.6)
3 month	25.0 (28.2)	6.3 (7.1)	30.7 (34.6)	26.6 (30.0)
4 month	22.3 (40.5)	7.7 (13.9)	25.1 (45.6)	14.5 (26.4)

Values in parentheses denotes percentage

Table 26. Incorporation of  $^{14}\text{C}$  in curcumin in the vegetative parts of turmeric during administration of  $1\text{-}^{14}\text{C}$  phenyl alanine

Sampling interval)	Plant part			
	Leaf (DPM)	Pseudostem (DPM)	Rhizome (DPM)	Root (DPM)
48 hour	100.0 (1.0)	161.5 (1.6)	1760.5 (17.9)	7815.4 (79.5)
1 week	147.0 (2.1)	142.5 (2.0)	243.4 (3.4)	6579.0 (92.5)
1 month	332.0(48.5)	99.3 (14.5)	125.6 (18.3)	127.2 (18.5)
2 month	133.9 (24.7)	143.2 (26.5)	97.5 (18.0)	166.3 (30.7)
3 month	133.4 (34.0)	81.1 (20.7)	45.8 (11.7)	131.6 (33.6)
4 month	68.5 (24.5)	76.3 (27.3)	36.1 (12.9)	98.8 (35.3)

Values in parentheses denotes percentage

Maximum incorporation was seen in roots after one week, with lower levels in leaves and rhizomes. In general, the incorporation of the label in the curcumin fractions was erratic and poor in the vegetative parts (Table 27). This shows that the alternate biosynthetic pathway suggesting malonyl Co-A as the immediate precursor can be ruled out.

#### *Sub cellular localization of PAL*

The localization of PAL, the rate limiting enzyme in curcumin biosynthesis using density gradient centrifugation and marker enzymes indicated its presence in the microsomal fraction with a minor fraction in the endoplasmic reticulum.

### 2. Characterization of drought tolerance in black pepper

(K. S. Krishnamurthy, S. J. Ankegowda and K. V. Saji)

#### *Screening*

In preliminary screening, 137 black pepper germplasm accessions were screened for drought tolerance in 4 sets of 45, 30, 30 and 32 accessions each. Some of the relatively tolerant accessions identified are Accs. 1380, 1387, 1410, 1423 and 1430.

#### *Intrinsic water use efficiency*

Intrinsic water use efficiency (A/g<sub>s</sub>) was measured in some of the tolerant and susceptible accessions identified during the previous year to understand the physiological

**Table 27.** Incorporation of 2-<sup>14</sup>C-malonyl Co-A in curcumin

Sampling intervals	Plant part			
	Leaf (DPM)	Pseudostem (DPM)	Rhizome (DPM)	Root (DPM)
1 week	84.0	412	53	1498
1 month	28.7	35.9	34	227.6
2 month	17.6	42.8	31.3	53.6
3 month	30.8	25.5	26.1	34.3
4 month	25.4	26.3	24.03	28.2
5 month	28.8	25.6	23.9	24.5
6 month	-	-	23.9	30.7

mechanism leading to water stress tolerance. In general, tolerant accessions showed higher water use efficiency compared to susceptible accessions after 6 days of stress induction. But at 3 days after stress induction, both tolerant and susceptible accessions had similar water use efficiency values. But the transpiration rate remained almost the same in both tolerant and susceptible accessions.

#### *Isozyme profiles*

Isozyme profiles of catalase, peroxidase and superoxide dismutase (SOD) were studied in 10 tolerant and 14 susceptible accessions identified during the previous year. Catalase showed single band (rm value of 0.14) irrespective of stress levels and genotype. Similarly, SOD showed three bands (rm values of 0.18, 0.34 and 0.47), which again did not differ either with stress levels or with the genotype. Peroxidase showed three isoforms (rm values of 0.12, 0.3 and 0.51). Here also, genotypic differences were not seen but the band with an rm value of 0.51 was generally seen under water stressed conditions.

### 3. Characterization of drought tolerance in cardamom

(S. J. Ankegowda and K. S. Krishnamurthy)

#### *Collection*

Three genotypes with high biomass and yield were collected from farmer's field from Kodagu District and one of the clones collected produced 5 kg wet cardamom capsule.

#### *Hybridization*

Four genotypes namely APG-243 (CCS-1), APG-244, APG-257 (Green gold) and APG-298 (IISR Avinash) were crossed with each other and seedlings were raised for further evaluation for higher yield and drought tolerance in the field.

#### *Evaluation of cardamom accessions*

Forty five genotypes were tested for relative water content (percent reduction over

control), specific leaf weight, stomatal count and membrane leakage. They recorded significant variation. Relative water content ranged from 16.95% to 30.76% with a mean of 22.95%. Specific leaf weight ranged from 3.55 mg/cm<sup>2</sup> to 6.9 mg/cm<sup>2</sup> with a mean of 5.47 mg/cm<sup>2</sup>. Stomatal count (60x) ranged from 7.8 to 14.38 with a mean of 9.94 per microscopic field. Membrane leakage ranged from 4.13% to 11.77% with a mean of 7.63%. Genotypes Mysore-2, Mysore-3, Vazhukka-1, Vazhukka-7, APG-343, APG-355, Malabar-13, Malabar-15 and Malabar-16 had low reduction in relative water content. APG-11, APG-13, APG-257, APG-298, APG-340, APG-341, APG-342, APG-349 and APG-355 had higher specific leaf weight. Hybrid APG-336, APG-337 and Mysore-7 had less stomatal count. APG-298, APG-346, APG-355, Malabar-12 and Malabar-15 had low membrane leakage.

Six accessions namely, Green Gold, Mysore-2, Malabar-18, APG-277 and APG-386 were planted in cement pots under rainout shelter for evaluation for drought tolerance.

Seventeen genotypes were analysed for delta <sup>13</sup>C using Isotope Ratio Mass Spectrometer (IRMS) at National Facility for Stable Isotope Studies in Biological Sciences at Department of Crop Physiology, University of Agricultural Sciences, Bangalore. Data indicated significant variation for <sup>13</sup>C discrimination. Delta <sup>13</sup>C values ranged from 20.54 to 22.36 with a mean of 22.27. Genotypes APG-298 (20.54) and APG-355 (20.64) recorded low discrimination values indicating higher water use efficiency. Genotypes APG-306 (23.365) and APG-339 (23.25) recorded higher delta <sup>13</sup>C discrimination indicating low water use efficiency.

#### 4. Physiological and biochemical basis for productivity in black pepper

(K. S. Krishnamurthy, B. Chempakam, K. Kandiannan, and Johnson K. George)

Metabolite partitioning, isozymes profiles and

gas exchange parameters were studied in five high yielding (Acc. 813, Acc. 1041, OPKm, HP-780 and HP-1411) and five low yielding (Accs. 840, 1120, 1157, 4132 and 5349) accessions of black pepper to understand the physiological and biochemical basis of productivity in black pepper.

#### Metabolite partitioning

Contents of total carbohydrates, reducing sugars, total phenols, amino acids and proteins were estimated in selected high as well as low yielders mentioned above. Carbohydrate content varied significantly among the accessions. In general, partitioning of total carbohydrates to roots was highest followed by leaf, petiole and stem. Leaves, stem and petioles of high yielders had lower level of carbohydrates than those of low yielders where as in roots, a reverse trend was observed (Table 28).

Analysis of reducing sugars revealed that high yielders had more reducing sugars in petioles and less in leaves compared to low yielders. Stem and root reducing sugar levels remained same among both high and low yielders.

**Table 28.** Total carbohydrates (%) in high and low yielding black pepper accessions

Accessions	Leaf	Root	Petiole	Stem
<i>High yielders</i>				
Acc. 813	7.34	10.90	3.29	5.88
Acc. 1041	6.46	10.78	4.46	5.18
OPKm	4.69	4.00	6.27	4.06
HP-780	4.44	5.43	5.41	3.50
HP-1411	9.55	12.32	4.26	5.28
<i>Low yielders</i>				
Acc. 840	11.76	11.71	9.71	9.78
Acc. 1120	9.87	9.23	10.16	7.50
Acc. 1157	6.78	8.88	6.48	12.28
Acc. 4132	6.76	6.61	6.70	9.33
Acc. 5349	4.58	7.55	2.73	4.82
CD (P<0.05)	1.46	1.62	1.23	2.17

Among different parts, root contained highest phenol level followed by leaves, stem and petioles. Leaves, roots and stem of high yielding accessions contained higher phenol content than those of low yielders (Table 29).

Protein content differed significantly among the accessions. Roots showed highest protein level followed by stem, petioles and leaves. Roots of low yielding accessions showed highest protein level where as reverse trend was observed in leaves and petioles. Stem showed highest amino acid level followed by leaves, roots and petioles. Both high and low yielders had similar level.

### *Isozyme profiles*

Isozyme profiles of peroxidase, polyphenol oxidase, malate dehydrogenase, esterase and acid phosphatase were studied in high and low yielding black pepper accessions.

Peroxidase had three prominent identical bands in the leaves of 4 high yielders among five, which were not conspicuous in low yielders. In roots, four identical bands (rm value of 0.05, 0.38, 0.57 and 0.62) were found in both high as well as low yielders.

**Table 29.** Total phenols (mg g fw<sup>-1</sup>) in high and low yielding black pepper accessions

Accessions	Leaf	Petiole	Root	Stem
<i>High yielders</i>				
Acc. 813	8.45	6.32	6.41	6.43
Acc. 1041	8.13	5.15	5.36	8.98
OPKm	8.33	5.25	10.61	4.55
HP- 780	8.57	6.06	10.91	9.88
HP-1411	5.51	4.68	12.47	4.65
<i>Low yielders</i>				
Acc. 840	7.41	3.77	8.81	4.75
Acc. 1120	7.16	3.37	5.78	6.16
Acc. 1157	6.41	3.95	7.72	4.35
Acc. 4132	6.90	4.33	6.49	7.40
Acc. 5349	8.73	5.54	7.49	12.63
CD (P<0.05)	1.87	1.56	2.10	2.34

Malate dehydrogenase showed two isoforms with rm values of 0.05 and 0.11 and acid phosphatase had three isoforms (rm values of 0.12, 0.33 and 0.42). The pattern remained same for both high as well as low yielders.

Similarly, polyphenol oxidase showed 4 bands of which rm values of 0.36 and 0.62 were generally found in high yielders.

### Externally Funded Project

#### Final Report

#### 1. ICAR: Elucidation of biosynthetic pathways of curcumin in turmeric

(B. Chempakam, K. Vasu and N. K. Leela)

#### Objectives

- To study the nature of precursors, intermediates and degradation products of curcumin so as to evolve a suitable biosynthetic pathway.
- To assay and localize the key enzymes involved in the biogenesis, based on the established pathway.
- Exploring the possibilities of utilizing the data generated from the scheme for establishing the pathways of biosynthesis of the active principles in other spices namely, pepper and ginger.

#### *Changes in curcumin, starch and secondary metabolites during plant growth*

This work was taken up to understand the level of pigments in the different vegetative parts of turmeric from the third month after sowing and its relative variation with respect to other major components namely, essential oil, oleoresin and starch.

Maximum content of curcumin was seen between 120 and 150 DAS (days after sowing), in the five varieties which decreased gradually and remained stable after 180 DAS. Essential oil content decreased gradually as the rhizomes developed. Higher concentration was seen in the first two stages (120 and 150 DAS). Gas chromatographic analysis of essential oil showed that in root

and rhizome, the major constituents are ar-turmerone and ar-curcumene (46.8% and 31.5%) respectively, while in leaf, the major component is  $\alpha$ - phellandrene (32.62%). In turmeric leaves also, curcumin and essential oil content were higher in the initial stages, which declined with the growth of the plant.

Oleoresin also showed a gradual decline during the maturity of the rhizome. The oleoresin in the early stages ranged from 17.5% to 24% in the immature rhizomes, while it ranged from 6.6% to 9.64% in the mature ones, accounting for about 50% – 70% of decline (Fig. 12) var. Prabha and Alleppey Finger Turmeric possessed higher oleoresin (24.01% and 23.9% respectively) in the initial stages. A gradual increase in starch was seen in all the five varieties during rhizome growth. Higher dry recovery was seen in var. Prabha, Prathibha and Alleppey Finger Turmeric (25.7%, 20.2% and 23.9% respectively) in the mature stage, while Suguna and Sudarshana had lesser dry recovery (11.9% and 13.2% respectively).

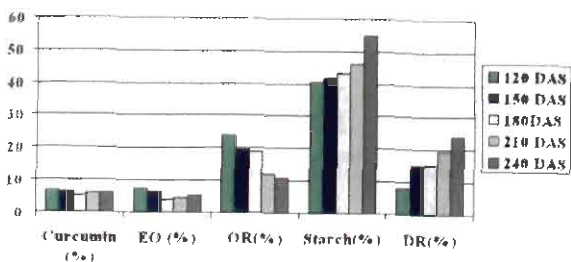


Fig. 12. Curcumin, essential oil, oleoresin, starch and dry recovery in turmeric rhizomes during development

#### Distribution of curcuminoids in rhizomes.

Curcumin (curcumin I), which forms the major portion of the curcuminoids, is distributed uniformly in mother, primary and secondary rhizomes only in the initial stage (120 DAS). As the rhizomes develop, the primary and secondary rhizomes contain higher proportion of curcumin (61.8–73.6%) as compared to mother rhizomes (46.4%). At full maturity, however, primary rhizomes had the highest content. This indicates that at later stages there was a lower production of

curcumin II and III, which are the two demethoxy forms. These compounds probably substantiate by providing the methyl acceptor to the final methylated form.

Curcumin II (demethoxycurcumin), which is structurally similar to bis demethoxy curcumin (curcumin III), except for an additional methoxy group ( $-\text{OCH}_3$ ) was maximum in mother rhizomes at 150 DAS. The secondary rhizomes also possessed a higher level at full maturity. In all other stages, the compound was at low concentration and varied from 11% to 24%, with highest in mother rhizomes and lowest in secondary rhizomes.

Curcuminoids in the pooled rhizome samples exhibited a similar pattern, where maximum levels of curcumin III were observed at 180 DAS. The only difference was that curcumin II and III are present in equal proportion, except at stage III where it was proportionately low as compared to curcumin I.

#### Studies on phenylalanine ammonia lyase (PAL)

##### PAL in early germination phase

PAL is the major rate limiting enzyme in phenylpropanoid metabolism and hence associated with curcumin biosynthesis. The activity was assayed in the early stages of rhizome development. For this, turmeric rhizomes from two varieties (Prathibha and Suguna) were sown and samples were taken at 15 day intervals to assess the PAL activity for a period of 3 months.

The activity was maximum at 120 DAS in rhizomes which declined afterwards (Table 30). The activity in roots showed a steady increase. The activity of PAL was very low in roots at 150 DAS, which gradually increased during the next intervals. In rhizomes and leaves, initial PAL activity showed an increase, which came down during the plant growth. In rhizomes, the activity decreased to about  $1/5^{\text{th}}$  of the initial value, whereas in leaves, a decrease of about 60% was seen.

**Table 30.** PAL activity in rhizomes, root and leaf at very early stages in turmeric

Plant part	PAL activity ( $\mu\text{M trans-cinnamic acid released/min/mg protein} \times 10^2$ )				
	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS
<i>Prabha</i>					
Rhizome	82.30	20.40	21.20	14.40	14.40
Root	6.80	8.11	21.80	25.38	28.40
Leaf	30.30	22.50	7.01	21.70	12.01
<i>Suguna</i>					
Rhizome	111.50	7.40	8.16	20.90	24.20
Root	3.38	3.71	5.32	10.61	9.44
Leaf	137.10	6.99	15.20	8.59	1.48

DAS- days after sowing

*PAL in low and high curcumin accessions*

PAL activity was seen in low and high curcumin accessions. A direct correlation exists with curcumin and PAL activity in low and high curcumin accessions, indicating a prime role for PAL in the biosynthesis of the pigment.

*Tracer studies*

The following three tracer incorporation studies using  $^{14}\text{CO}_2$ ; root absorption studies with 1- $^{14}\text{C}$ -phenylalanine and root absorption and incorporation in curcumin using 2- $^{14}\text{C}$ -malonyl Co-A experiments using tracers were conducted in turmeric.

Table 31 shows the incorporation as DPM of  $^{14}\text{C-CO}_2$  in the curcumin extracted from roots, rhizomes, pseudostem and leaves, at various

**Table 31.** Incorporation of  $^{14}\text{C-CO}_2$  in curcumin among the vegetative parts of turmeric

Period	Root (%)	Rhizome (%)	Pseudostem (%)	Leaf (%)
24 hour	24.30	11.3	15.4	49.00
48 hour	24.90	14.6	23.4	37.16
96 hour	22.90	22.7	11.73	42.58
1 week	25.01	32.8	41.78	0.37
1 month	67.52	13.9	11.42	7.28
2 month	35.68	29.5	16.80	18.02
3 month	66.12	13.3	17.95	2.61
4 month	83.10	4.88	5.04	6.98
5 month	66.10	17.8	5.15	10.96

intervals. Maximum incorporation of the label was found in roots from one month onwards.

Turmeric rhizomes were sown in polybags. One month old seedlings of var. Prathibha of uniform size were selected for the studies. The roots of the seedlings were immersed in a trough containing  $^{14}\text{C}$ -phenylalanine for 24 h and then replanted in plots. Samples were analysed for the incorporation of the

**Table 32.**  $^{14}\text{C}$ - Incorporation of curcumin in root, rhizome pseudostem and leaf after exposure to  $^{14}\text{C}$ - phenylalanine

Period	Root (%)	Rhizome (%)	Pseudostem (%)	Leaf (%)
48 four	52.38	39.32	10.17	9.86
1week	44.09	53.91	23.57	14.42
1month	0.85	2.18	23.69	35.73
2 month	1.11	0.98	12.72	15.50
3 month	0.66	0.81	13.42	15.06
4 month	0.88	2.81	16.42	9.17

labeled compound as phenolic acids and curcumin. Same procedure was followed for the incorporation studies using  $^{14}\text{C}$  malonyl Co-A.

Maximum incorporation of  $^{14}\text{C}$ -phenylalanine in curcumin was seen in the early period (1 month) in the leaf (Table 32). Simultaneous incorporation in both components indicates a direct role for phenylalanine as initial precursor. Translocation and incorporation of  $^{14}\text{C}$

malonyl Co-A is poor and erratic and hence initial precursor as acetate can be ruled out.

### Summary

- PAL the major enzyme, which initiates the series of reactions leading to curcumin synthesis, was studied during the early germination phase. The activity was maximum in leaves as compared to roots, rhizomes and pseudostem, indicating that the conversion of phenylalanine to cinnamic acid mostly takes place in the leaves
- Studies on the localization of PAL activity in various cell fractions showed maximum activity in microsomal fraction
- GC-MS studies of the essential oil from rhizome, root and leaf indicated the major compounds as ar-turmerone and ar-curcumene in rhizome and root and  $\alpha$ - phellandrene and terpenolene as the major ones in leaf oil.
- Majority of the phenolic acids identified in the leaf are coumaric, caffeic and ferulic acids. These phenolic acids are intermediate precursors in the proposed pathway of curcumin biosynthesis.
- A direct correlation exists with curcumin and PAL activity in low and high curcumin accessions, indicating a prime role for PAL in the biosynthesis of the pigment.
- Studies using labeled  $\text{CO}_2$ , labeled  $^{14}\text{C}$ -phenylalanine and  $^{14}\text{C}$ -malonyl Co-A to see the incorporation of the intermediary precursors have been completed.

## V. Mega Project: Value addition and post harvest processing of spices

(Project Leader: T. John Zachariah)

### 1. Quality evaluation in spices

(T. John Zachariah and N. K. Leela)

#### Black pepper

Black pepper hybrids and collections were analyzed for oil, oleoresin and piperine and the high quality accessions are listed in Table 33.

The chemical quality (oil, oleoresin and piperine) of pepper grafts (*P. colubrinum* as rootstocks) and non-grafts of black pepper, Kutching, Panniyur-1, 2, 3, 4, 5, Panchami, Poonjaramundi, Kuthiravally, Sreekara, Subhakara, Kottanadan were compared. There was no variation in quality between grafts and non-grafts.

**Table 33.** High quality black pepper accessions

Accession	Essential oil (%)	Oleoresin (%)	Piperine (%)
HP-813	3.7	11.0	4.2
C-950	3.3	8.0	3.2
C-1041	3.7	9.2	3.0
P-24	3.3	9.6	4.1
HP-150	-	12.2	3.9
HP-1662	-	13.0	5.0
HP-4052	-	12.4	3.0
HP-4187	-	13.2	3.1
<i>P. argyrophyllum</i>	3.4	11.5	-
<i>P. attenuatum</i>	3.0	9.7	-

#### Cardamom

Seventy seven germplasm accessions of cardamom were evaluated for essential oil and GC profile of oil and the high quality accessions of cardamom are listed in Table 34. The oil content ranged from 5.2% to 8.5%. All the 77 accessions had about 1.6% pinene. Sabinene ranged from 1.8% to 4.0%; myrcene from 1.6% to 4.5%; limonene from 3.1% to 8.8%. 1, 8-cineole and  $\alpha$ -terpinyl acetate are

**Table 34.** High quality cardamom accessions

Accession	Oil (%)	1,8-cineole (%)	$\alpha$ -terpinyl acetate (%)
APG-246	8.4	29.6	39.2
APG-248	8.5	33.0	36.3
APG-352	8.3	26.2	32.7
APG-357	6.6	31.9	42.5
APG-365	6.6	27.2	43.8
APG-378	8.1	33.7	36.3
Mysore-2	7.5	22.3	41.0
Malabar-27	7.0	43.4	34.4
OP-N-19	7.6	31.8	34.6

the key constituents, which determine the flavour of cardamom oil. The ratio of these constituents determines the overall flavor note of cardamom oil. 1, 8-cineole imparts a camphoraceous note and  $\alpha$ -terpinyl acetate imparts sweet floral note. 1, 8-cineole content among the accessions varied from 22% to 43%.  $\alpha$ -Terpinyl acetate content varied from 26% to 43%.

#### Ginger

Fifty ginger accessions were evaluated for oil, oleoresin and crude fibre content and the high quality accessions are listed in Table 35. Essential oil content range from 0.6% to 2.0% and oleoresin range from 2.5% to 5.3%. Accessions 135, 156, 201, 233, 270, 480, 507 and 515 had crude fibre content below 2.5%

**Table 35.** High quality ginger accessions

Accession	Oil (%)	Oleoresin (%)	Crude fibre (%)
Acc. 428	1.6	3.0	3.0
Acc. 512	1.3	5.3	4.3
Acc. 555	2.0	4.9	5.7
Acc. 558	1.5	4.6	6.5



and accessions 195, 452, 476, 486, 535, 551, 555 and 558 contained more than 5% crude fibre. It was observed that accessions with high oleoresin contained more crude fibre. Accessions 176, 184, 297, 468, 507 and 531 had more than 40% zingiberene, the main constituent of ginger flavour in the oil. Acc. 486 contained highest limonene and linalool in the oil.

Ginger collection from Achankovil area (Acc. 652) contained 3.2% oil with 59.5% zingiberene, 11% geraniol and 5.1% limonene. Acc. 653 contained 3% oil with 44% zingiberene, 23.8% geraniol and 7.1% limonene. The oil profile of these accessions are remarkably different compared to regular ginger accessions. Contrary to yellowish tinge of regular ginger oils these accessions had colorless or white oil.

### *Turmeric*

Among the 400 turmeric accessions evaluated for quality Accs. 124, 201, 227, 264, 295, 435, 544, 547, 548, 575 and 593 had more than 12% oleoresin. Only three accessions Acc. 240, 592 and 593 contained 5.5 % curcumin. Acc. 103, 114, 334, 413, 422, 540, 547, 548 and 575 had more than 5% essential oil.

### *Cassia*

Bark of *Cinnamomum burmanii* (Padang cassia) contained 2.8% oil and 11.8% oleoresin. *Cinnamomum loureirii* (Vietnam cassia) contained 3.3% oil.

## Final Report

### 1. Harvesting and processing techniques in spices

(P. Heartwin Amaladhas, T. John Zachariah and A. Kumar)

#### *Objectives*

- To survey the indigenous technologies followed in drying and processing of various spices.
- To compare the quality aspects of spices processed by traditional method with the improved mechanical methods.

- To study the drying characteristics of nutmeg and optimize the drying parameters.
- To study the death time characteristics of wilt causing bacteria in ginger and development of heat treatment unit.
- To develop a thresher for black pepper suited to small and marginal farmers.
- To develop a cleaning and grading system for black pepper.

### *Dehydration*

#### *Mace and nutmeg*

Hot air drying in agricultural waste fueled dryer and hot sand drying methods were followed to dry mace and nutmeg. In hot air drying, the temperature was maintained in the range of 55 to 65 °C. Both mace and nutmeg were spread in a single layer over the wire mesh. It was observed that drying of mace took 4 h while that of nutmeg took 16 h. The dry recovery of mace and nutmeg were 33.1% and 70.6 % respectively. In the hot sand drying method, a sand bed was placed over a wood fueled stove and mace and nutmeg were spread over it. It is one of the traditional methods followed for drying of nutmeg where the sand bed provides a continuous heat supply to the produce. In this method, control over the temperature of drying is limited. Drying was over in 3.5 h for mace and 14 h for nutmeg. Analysis of quality of air dried and hot sand dried mace and nutmeg showed that the quality was better in hot air drying.

Hot air drying of mace took 4 h while blanched (for 2 min in 75 °C water) mace required only 3.5 h for drying. The dry recovery of hot air dried and blanched mace were 37.8% and 37.1%, respectively. Analysis of quality of dried mace showed that fresh mace had a lycopene content of 121.92 mg/100 g which after drying changed to 148.98 mg/100g and 182.23 mg/100 g, respectively, for hot air dried and blanched mace. Blanched mace gave 23% more colour

and more colour stability than hot air dried mace. Volatile oil and oleoresin contents of hot air dried and blanched mace were comparable.

#### Piper chaba

*P. chaba* at three maturity levels namely mature (turning green to orange), ripe (completely red) and over ripe (soft and red) were dried in both sun and dryer. Sun drying took 5 days while hot air drying took only 8 h for complete drying. The dry recovery was between 30 and 34 % in both methods of drying. Dry recovery was found to be highest in over ripe sample because of its low moisture content at the time of harvesting. Drying of *P. chaba* in hot air at 60 to 65 °C at three stages of maturity indicated that it took 9 h to attain a safe moisture level. The dry recovery at mature, ripe and over-ripe stages were 29.4%, 30.1% and 30.2%, respectively, compared to 71.8%, 71.6% and 71.4%, respectively in the fresh state. The moisture, oil and oleoresin contents in these stages were also estimated before and after drying.

#### Cassia

Effect of sun drying and hot air drying (50 °C) on cassia were studied. Sun drying took 1-2 days while hot air drying took only 3 h. The dry recovery of cassia after sun drying and hot air drying were 34.0% and 33.7%, respectively.

#### Storage of mace

Mace was stored in PET, polythene and polypropylene containers and the changes in quality characteristics like moisture content, volatile oil, oleoresin and lycopene contents were analysed. The moisture content increased during storage while volatile oil, oleoresin and lycopene contents decreased. After 3 months of storage, the lycopene content degraded by 68% to 86% in hot air dried mace and 66% in blanched mace.

#### Aerated steam treatment of ginger rhizomes

Ginger rhizomes infected with soft rot pathogens were treated using aerated steam

at 46 and 51 °C for 15 min and 30 min, for standardizing optimum conditions for treatment. After treatment, the rhizomes were treated with biocontrol agents (*Trichoderma* sp. and *Pseudomonas* sp.) and planted in the field and the plant population and yield were recorded. Aerated steam treatment at 51 °C for 15 min was most effective in controlling the disease.

#### Development of black pepper thresher

Two models of black pepper threshers (drum type and funnel type) were developed in collaboration with Tamil Nadu Agricultural University (TNAU), Coimbatore. The threshing capacities of drum type and funnel type threshers were 150 and 75 kg per hour, respectively with a threshing efficiency of 99.6% and 98.5%, respectively. Two commercially available models namely, Vivega hand-operated and Vivega power-operated threshers were also evaluated for their performance.

#### Externally Funded Project

##### Final Report

#### 1. NATP: Value addition and quality enhancement of selected spices

(*P. Heartwin Amaladhas, B. Chempakam and T. John Zachariah*)

##### Objectives

- To develop a mechanical dryer for black pepper (50 kg per batch)
- To develop a mechanical thresher for black pepper.
- To fabricate an equipment for cleaning and grading of pepper.
- To develop a pilot plant for the production of white pepper.
- To refine existing technologies in the garbling of cardamom.
- To develop an on farm dryer for chilli suitable for centralized drying.

##### Achievements

- The engineering properties of three

varieties of black pepper, namely, IISR Shakthi, Panniyur and Karimunda were determined. The dimensions, sphericity and berry/spike ratio was highest for Panniyur, while IISR Shakthi had the highest bulk density.

- Hot air drying of black pepper using agricultural waste fueled type dryer took 9 h and the dry recovery was found to be 36.4%, 32.3% and 33.3% for IISR Shakthi, Panniyur and Karimunda respectively.
- Sun drying, which took 4–5 days, also resulted in a higher dry recovery for P-24. However, hot air dried pepper resulted in a reduction in the oleoresin and volatile oil content.
- Among four types of pepper thresher evaluated, TNAU drum type thresher with a threshing capacity of 150 kg/h was found to have a threshing efficiency of 99.6% (Fig. 13).

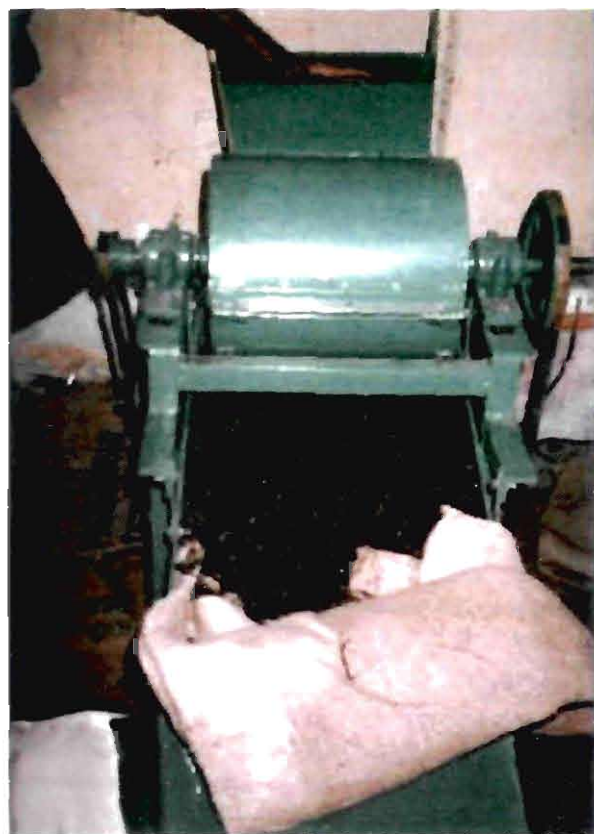


Fig. 13. Black pepper thresher

- The cleaner-cum-grader developed by the lead centre was evaluated for its effectiveness at different rpm.
- To refine the existing methodology for the production of white pepper, pit method was found to be more advantageous with less water requirement and less pollution retting method. The method consisted of placing the berries in polybags at 60 cm below the soil surface and moistening once in 3 days for a total of 14 days.
- The natural microflora associated with the surface of black pepper berries were isolated and these isolates were tentatively identified as *Bacillus* spp.

## 2. NATP: Integrated technologies for value addition and post harvest processing in palms, spices and tropical tuber crops (T. John Zachariah, B. Chempakam, N. K. Leela and S. Devasahayam)

- Indigenous technologies available in despiking of pepper, cleaning and grading of pepper, curing of cardamom and drying of nutmeg and mace were documented.
- Various pepper threshers available were evaluated. A unit with a capacity to despice 150 kg/h green pepper with 99.6% efficiency with an approximate cost of Rs.15,000/- was identified as the most ideal one.
- A copra drier was modified and found suitable to dry mace in about 3 h without loss of colour and chemical quality.
- The main microorganism involved in white pepper fermentation was identified as *Bacillus subtilis*.
- A zero energy chamber was fabricated to store fresh ginger. Fresh ginger stored in a wooden box in the chamber showed weight reduction of only 23% compared to 39% in wooden box alone and 50% in open condition.

- Storing dry ginger in PET jars with leaves of *Azadiracta indica*, *Glycosmis cochinchinensis* and *Clerodendron infortunatum* prevented attack of storage pests such as *Lasioderma serricorne*.
- Pepper, ginger, turmeric, nutmeg and mace can be successfully stored under vacuum in aluminium foil, which preserve the material from moisture reabsorption and maintain the physical and chemical quality.
- Super critical extraction of pepper and ginger oil using liquid carbon dioxide indicated that compared to hydrodistillation the former possess more fruity and sweet aroma. However, GC profile of the two reveal that super critical extraction reduces recovery of many low boiling compounds.

## VI. Mega Project: Production of nucleus planting materials of improved varieties of spice crops

(Project Leader: C. K. Thankamani)

### 1. Production of nucleus planting materials of improved varieties of spice crops

(C. K. Thankamani, P. A. Mathew, K. Kandiannan and S. J. Ankegowda)

Black pepper rooted laterals (50,000) (Fig. 14), turmeric seed rhizomes (3 t), ginger seed rhizomes (2 t), cardamom seedlings (25,799), cardamom suckers (1226), 649 garcinia grafts and nutmeg grafts (6000) were produced and distributed to farmers and other agencies.



Fig. 14. Production of nucleus planting material of high yielding black pepper varieties

## VII. Mega Project: Identification, characterization and development of diagnostics against pests, pathogens and nematodes of spice crops

(Project Leader: M. N. Venugopal)

### 1. Investigations on stunted and phyllody diseases of black pepper

(A. Ishwara Bhat, S. Devasahayam, M. N. Venugopal and R. Suseela Bhai)

Three districts of Karnataka (Dakshina Kannada, Hassan and Uttara Kannada) and four districts of Kerala (Idukki, Kannur, Kasaragod and Kozhikode) covering 216 black pepper gardens were surveyed for the presence of stunted disease and associated insect fauna. The incidence of the disease was highest at Idukki District of Kerala (Table 36). In Kerala except for four locations (Kodathai, Mulleria, Santapara and Thodupuzha), the incidence of viral disease was noticed in all the locations surveyed. No incidence of viral disease was noticed in any of the plantations surveyed in Dakshina Kannada District of Karnataka while only one plantation in Uttara Kannada District of Karnataka showed the presence of the disease. In Hassan District, Belur Taluk had a higher disease incidence compared to other taluks surveyed. In general disease incidence and severity was higher in black pepper plantations situated at higher altitudes of Kerala. Two major disease symptoms were noticed in the areas surveyed. Typical symptoms of stunting such as mosaic mottling on leaves, reduced leaf size, brittle leaves, reduced internodal length leading to stunting of the whole plant were the most prominent symptoms observed in majority of the black pepper plantations in Idukki and Wyanad districts of Kerala. The other symptoms, which was observed mainly in black pepper plantations grown at coastal plains, included chlorotic flecking, bright yellow mottling along the veins coupled with characteristic curling of leaves. Reduction in spike length and poor filling of spikes were observed in most of the diseased vines. Masking of symptoms (especially in older

leaves) during monsoon and winter months were seen in many of the affected black pepper vine. Symptoms were best exhibited in the affected plant during March to May. All cultivars and improved varieties including hybrids were found susceptible to the diseases. Vines of all ages raised on all kinds of standards were also found affected by the diseases. Among the several weeds found in and around black pepper plantations, a few of them showed typical viral like symptoms, which might act as potential alternate hosts for the virus (Table 37). Though 12 species of insects were collected from diseased vines no species was specifically associated with diseased vines (Table 38).

Table 36. Incidence of viral diseases of black pepper in various districts of Karnataka and Kerala

State /District	Range of incidence of disease (%)	Mean incidence (%)
<i>Karnataka</i>		
Dakshina Kannada	0	0
Hassan	0-20	5.2
Uttara Kannada	0-2	0.4
<i>Kerala</i>		
Idukki	0-78	29.4
Kannur	2-42	19.5
Kasaragod	0-53	18.9
Kozhikode	0-33	10.7

When the collected isolates were subjected to direct antigen coated (DAC) ELISA using antisera to different viruses, majority of the isolates reacted with antiserum to either CMV or banana streak badnavirus (BSV) while a few of the isolates reacted with both the antisera indicating the involvement of two viruses belonging to the genera *Cucumovirus* and *Badnavirus* in the disease. Majority of the isolates from Idukki and Wyanad districts of

Table 37. Weed flora with viral like symptoms in the plantations surveyed

Family	Genus/species	Symptoms observed
Amaranthaceae	<i>Amaranthus viridis</i>	Yellowing, dark pin head size lesions on leaves
Araceae	<i>Colocasia esculanta</i> (L.) Schott	Mosaic
Asteraceae	<i>Ageratum conyzoides</i>	Yellow mosaic and leaf curl
	<i>Chromolaena odorata</i> (L.) R.M. King & H. Rob	Leaf curl
	<i>Sonchus oleraceus</i> L.	Mosaic
	<i>Synedrella nodiflora</i> (L.) Gaertn	Leaf curl and stunting of plant
Euphorbiaceae	<i>Euphorbia geniculata</i>	Leaf curl
Lamiaceae	<i>Leucas aspera</i> (Willd.) Link	Marginal leaf curl
Oxalidaceae	<i>Oxalis corniculata</i> L.	Leaf curl
Rubiaceae	<i>Knoxia corymbosa</i> Willd.	Leaf curl

Table 38. Insects associated with viral disease affected black pepper vines

Family	Genus/species
Aleyrodidae	<i>Bemisia tabaci</i> (Genn.)
Aphididae	<i>Toxoptera aurantii</i> (B.de F.)
Pseudococcidae	<i>Planococcus</i> sp
Diaspididae	<i>Lepidosaphes piperis</i> Green
Phlaeothripidae	<i>Liothrips karnyi</i> Bagn.
Chrysomelidae	<i>Longitarsus nigripennis</i> Mots
Tortricidae	<i>Cydia hemidoxa</i> Meyr
Geometridae	<i>Synegia</i> sp.

Kerala reacted with CMV antiserum while majority of the samples from remaining locations reacted with BSV antiserum indicating the involvement of badnavirus with the diseased vines collected from these locations.

The black pepper isolate of CMV maintained on *Nicotiana benthamiana* was transmitted from diseased to healthy *N. benthamiana* plants using the aphid, *Pentalonia nigronervosa* with five minutes acquisition access and 12 h inoculation access periods. The inoculated showed typical symptoms of the disease within 15 days after inoculation. Transmission studies from infected black pepper to healthy black pepper using *P. nigronervosa* and another aphid species found colonizing black pepper i.e. *Toxoptera aurantii*

are underway. Similarly, transmission of badnavirus from diseased to healthy black pepper using root mealy bug (*Planococcus* sp. and *P. citri*) are also under progress.

## 2. Investigations on spike shedding of black pepper at high altitudes

(M. Anandaraj, M. N. Venugopal, K. S. Krishnamurthy, V. Srinivasan, K. Kandianan, R. Ramakrishnan Nair and S. J. Ankegowda)

### Etiology and management of spike shedding in black pepper

Observations recorded on severity of spike shedding in various crop combinations, input management and light profiles indicated that the incidence varied from 9% to 87% in different field situations. Highest spike shedding was noticed in the rainfed blocks and heavily shaded conditions (Table 39).

### Epidemiology

Monitoring of disease in Panniyur-I under different field conditions revealed that incidence commenced in the fourth week of June and reached its peak during August. The infection synchronized with the production of new flush and delayed emergence of spikes. Anthracnose infection declined after recession of monsoon and maturity stage of leaves and spikes. The spike

**Table 39.** Anthracnose incidence in different cropping system

Location	Situation /Crops	Disease index on laterals (%)	Spike infection (%)	Fallen spikes (%)	Spike shedding (%)
Ashoka Estate	Regulated shade	10	13	2	9
Ashoka Estate	Partial shade	23	37	21	53
Ashoka Estate	Shade	47	78	30	83
Laxmi Estate	Cardamom + pepper	52	62	28	74
Laxmi Estate	Coffee* + Pepper	19	23	12	16
Laxmi Estate	Coffee** +Pepper, irrigated	39	34	24	42
Chettalli	Regulated shade, rain fed	41	23	14	c
Udathmotte	Coffee* + Pepper, irrigated	26	39	19	41
Boikeri	Coffee* + Pepper, irrigated	21	18	13	24
Boikeri	Coffee** + Pepper, irrigated	27	34	16	32
Makkandur	Coffee** + Pepper, irrigated	72	83	33	87

\*Robusta ; \*\* Arabica

infection varied from 13.0% to 83.4% and highest infection was recorded in the plants under high shaded conditions. Fallen spikes revealed 33% anthracnose infection and the rest had only female flowers instead of bisexual flowers.

#### **Management of anthracnose**

In the second year of disease management trial, the application of fungicides and biocontrol agent was carried out in two rounds during early monsoon and mid monsoon periods. The fungicides were tried as foliar spray in twelve separate treatment combinations. Bordeaux mixture (1%) was superior over other fungicides and biocontrol agent (*P. fluorescens*) with mean green yield of 3.6 kg/plant and 29% disease index compared to only 0.78 kg green yield/plant and 61% disease index in untreated control. Application of *P. fluorescens* as spray and drench during premonsoon and midmonsoon period is also effective in management of anthracnose. The treated plants had 2.34 kg green yield/plant with 35% disease index. The application of biocontrol was on par with many standard fungicides like Carbendazim, Hexaconazole, Mancozeb, Zineb and 0.5% Bordeaux mixture (Table 40).

#### **Field reaction of pepper to anthracnose**

Anthracnose infection was recorded in 11

varieties and 14 popular cultivars. Among the varieties Panniyur-5, Panchami and Subhakara showed field tolerance to anthracnose infection and Panniyur-1 and 3 were highly susceptible. Among 14 cultivars Chomala, Thevanmundi, Karimunda, Chettalli selection, Aimirian and Arakalamunda showed field tolerance to anthracnose.

#### **Bisexual flower status in relation to light profiles**

Light availability at different heights of shade trees, pepper vine and different levels of shade was measured by using lux meter. Lux reading was recorded in open place, under filtered shade of shade tree with pepper and natural conditions. Ten readings were recorded for each observation for four days in February 2004 from 9.30 am to 10.30 am. Average lux reading in open (71212.9 lux), filtered light in silver oak (17784.2 lux) and mutual shade (4656.9 lux) in silver oak with pepper were recorded. Amount of light intercepted by silver oak was 75% and filtered 25% and unused light under mutual shade of shade tree with pepper was only 6.5%. Amount of light available in palwan (*Erythrina* sp.) with pepper system recorded 68502.5 lux in open condition; filtered 6547.5 lux and mutual shade was 3280.5 lux. The



light intercepted by palwan was 90% and unused light under mutual shade of shade tree with pepper was only 5%. 97.8% of the flowers in Panniyur-1 showed bisexual status under irrigated and exposed conditions compared to only 3.9% bisexual flowers in rain fed and heavily shaded conditions. Predominance of female flowers and lack of pollination in the rain fed, shaded and delayed emergence and anthracnose infection are the major reasons for spike shedding in high altitudes (Table 41).

#### *Effect of phytohormones, potash, zinc and boron on spike shedding*

Phytohormones like NAA (50 ppm), GA (20 ppm) and kinetin (BAP 20 ppm), potash, zinc and boron were tried to study their effect on spike shedding. Two rounds of hormones and nutrients were applied as foliar spray during spike initiation and fruit development stages. The spike shedding varying from 7 to 12% in the treatments compared to 19% in the untreated irrigated block. There were marked differences in the number of spikes/1m canopy height of canopy, total mean green yield and number of spikes to make 1 kg (Table 42).

#### *Impact of irrigation on spike shedding*

Many irrigation trials were taken up in hot spots of spike shedding. The trials were conducted purely as farmers' participatory programme under coffee based pepper cropping system with Panniyur-1. Four to five basin irrigation @ 50 litre/plant at an interval of 7-10 days were given to black pepper plants. The basin irrigation was followed by shade regulation of support trees to provide minimum light exposure of 6,500-12,000 lux under cloudy monsoon conditions. The basin irrigation was started in March 4 to 2 week of April and continued up to mid May. Irrigation had tremendous impact in enhancing early spiking, maturity of laterals, high percentage of bisexual flowers and better setting compared to rainfed blocks of Panniyur-1 in respective locations (Table 43)

#### *Causes for spike shedding*

Based on the above studies it is concluded that predominance of female flowers and lack of pollination under unregulated shade, delayed emergence in rain fed conditions, synchronization of flushing at vulnerable stage of crop to anthracnose infection are the main reasons for spike shedding in high altitudes.

**Table 40.** Effect of fungicides and *Pseudomonas fluorescens* on anthracnose incidence

Treatment		Disease index (%)	Yield/plant (kg) green	No. of spikes/kg
1 <sup>st</sup> round	II <sup>nd</sup> round			
Bordeaux (1%)	Bordeaux (1%)	29	3.60	144.0
Bordeaux (0.5%)	Bordeaux (0.5%)	34	2.16	177.5
Bordeaux 1%	Hexaconazole	30	2.10	158.3
Hexaconazole	Hexaconazole	27	2.00	156.3
Hexaconazole	Bordeaux (1%)	19	2.20	158.8
Carbendazim (0.2%)	Carbendazim (0.2%)	18	2.30	151.0
Mancozeb (0.25%)	Carbendazim (0.2%)	23	2.00	158.8
Carbendazim (0.2%)	Mancozeb(0.25%)	26	2.20	146.8
Mancozeb (0.25%)	Mancozeb(0.25%)	23	2.20	168.3
Zineb(0.25%)	Zineb (0.25%)	29	1.70	164.8
<i>P. fluorescens</i>	<i>P. fluorescens</i>	35	2.34	171.0
Potassium phosphonate	Potassium phosphonate	29	1.80	151.5
Control		61	0.78	149.8
CD (< 5%)			0.23	13.11

Table 41. Bisexual flower status in Panniyur-1 under different exposure levels

Treatment	Light availability	Bisexual flowers (%)
Panniyur-5	10,000 – 12,000 lux	100.00
Panniyur-1, 70% shade	6,547 lux	7.18
Panniyur-1, heavy shade	3,280 lux	3.9
Panniyur-1, Arabica coffee exposed	9,000 – 10,000 lux	83.57

### Management

Irrigation of black pepper vines 4–5 times at an interval of 5-7 days @ 40–50 plant commencing from 22 March, followed by shade regulation of support trees to provide minimum 7,500–10,000 lux light under cloudy condition is optimum for managing spike shedding in high altitudes. The irrigation coupled with recommended phytosanitary, prophylactic and nutrition management practices are necessary for holistic management of spike shedding and anthracnose in high altitudes.

### 3. Studies on bacterial wilt of ginger

(A. Kumar and R. Suseela Bhai)

#### *Changes in the microbial population on rhizome upon solarization*

##### *Changes in total microbial population*

Changes in the microbial population on rhizome upon solarization for one and two hours are presented in Table 44. Fungal and bacterial enumeration using standard plate

count technique was done immediately after solarization. One hour of rhizome solarization for 1 h and 2 h has recorded a rhizome temperature of 39.6 °C and 2 h of solarized rhizome is 50.0 °C respectively (Table 44). Over 50% reduction in the microbial population was recorded due to rhizome solarization. It is also interesting to note that the vascular microflora is much more vulnerable to heat than the outer surface as the reduction in microflora is more than 65% in vascular tissues.

##### *Changes in the specific microflora on/in rhizomes*

Ginger rhizomes collected from bacterial wilt affected fields were exposed to sunlight for 1 and 2 h from 9.00 am to 11.00 am on a bright sunny day. The rhizome temperature recorded were 39.6° C and 50.0° C after one and two hours respectively. Surface washings and vascular extracts obtained from the solarized and unsolarized rhizomes were subjected to serological assay for detection of

Table 42. Effect of hormones and nutrients on spike shedding and green yield

Treatment	Weight (kg)*	Spikes/1m canopy	Spikes/1kg	Spike shedding (%)
NAA	15.81	550	146	9
Kinetin	14.81	494	156	12
GA	15.04	597	161	10
NAA+GA+kinetin	13.60	551	152	12
NAA+GA+kinetin +K+Bo +Zn	15.3	522	146	11
MOP+Zn+Bo+NAA	19.0	705	140	8
MOP	20.8	742	150	7
Zn+Bo	15.0	551	147	12
Control	11.9	464	183	19

\*Green berries

Table 43. Impact of irrigation on spike shedding

Location	Crop combination	Situation	Spikes/m canopy
Hosakeri	Coffee* + Pepper	Irrigated	236
Hosakeri	Coffee* + Pepper	Rain fed	50
Sandalkad	Coffee** + Pepper	Irrigated	143
Sandaikad	Coffee** + pepper	Rain fed	39
Halery	Coffee* + Pepper	Irrigated	114
Halery	Coffee** + Pepper	Rain fed	63
Halery	Cardamom + Pepper	Rain fed	39
Boikeri	Coffee* + Pepper	Irrigated	194
Boikeri	Coffee** + Pepper	Irrigated	71
Boikeri	Coffee** + Pepper	Rain fed	48
Boikeri	Cardamom + Pepper	Irrigated	128
Ashoka	Cardamom + Pepper	Irrigated+Shade	19
Ashoka	Cardamom + Pepper	Irrigated+Shade	19
Ashoka	Coffee** + Pepper	Irrigated+Partial shade	31
Ashoka	Coffee** + Pepper	Rain fed regulated shade	464
Ashoka	Coffee** + Pepper	Rain fed	14
Udathmotte	Coffee* + Pepper	Irrigated	212
Udathmotte	Coffee* + Pepper	Rainfed	128

\*Robusta ; \*\* Arabica

*R. solanacearum* using DAS-ELISA. The results of the colour reaction as absorbance values at 405 nm are presented in Tables 45 and 46. Among the different samples positive reaction could be noted only from three locations namely, Kenichira, Poothady and Pulpally as their absorbance values are more than three times of that of negative control value. Interestingly extracts obtained from 2 h solarized rhizomes tested negative for *R. solanacearum* in these locations. The result clearly indicates that the rhizome

solarization has got detrimental effect on the survival of the bacterium on the rhizome and also in the vascular tissues.

#### Development of rhizome bacterization

In order to develop a suitable rhizo-and endo bacterial consortium for management of bacterial wilt in field, systematic isolation experiment was carried out to isolate resident bacteria from ginger. The endophytic bacterial isolates were isolated from ginger pseudostem. Briefly, cut ends of surface

Table 44. Changes in microbial population of ginger rhizome upon rhizome solarization

Time of solarization	Duration of solarization (h)	Rhizome temperature (°C)	*Percent reduction over control			
			Bacterial population		Fungal population	
			Surface	Vascular	Surface	Vascular
0.00	0	27.6	-	-	-	-
9 to 10 am	1	39.6	55.9	73.6	49.1	66.6
9 to 11am	2	50.0	84.2	92.0	79.7	91.5

\*Mean of six locations

**Table 45.** Changes in the population of *Ralstonia solanacearum* in ginger rhizomes during rhizome solarization as detected by DAS-ELISA

Time of solarization	Duration of solarization (h)	Rhizome temperature(°C)	A 405 Values*					
			Vadakkanad	Poothady	Kenichira	Beenachy	Ambalavayal	Pulpally
0.00	0	27.6	0.02	0.01	0.34	0.037	0.01	0.81
9 to 10 am	1	39.6	0.02	0.0	0.04	0.01	0.08	0.35
9 to 11 am	2	50.0	0.06	0.01	0.01	0.02	0.01	0.19
Negative control					0.04			0.28

\* Mean of two replicates, Absorbance was read at 405 nm 1 h after adding the substrate solution

sterilized ginger pseudostem were subjected to centrifugation at 10000 rpm at 4 °C for 30 minutes. The pellets thus obtained were plated directly on to nutrient agar medium. After sufficiently incubating the plates, the colonies were isolated and preserved in - 80 °C for long-term preservation as glycerol stocks

The isolated bacteria (21) was subjected to morphological characterization on 2, 3, 5 triphenyl tetrazolium chloride amended nutrient agar for discriminating the colonies. The details of the isolates are furnished in Table 47.

Twenty bacterial isolates obtained from bacterial repository was evaluated against *R. solanacearum* *in vitro*. Of the twenty isolates screened, 8 are found to inhibit the colony development as indicated by their reduced colony size, morphology as well as accumulation of green pigments. Isolates IISR-10, IISR-15, IISR-26, IISR-38, IISR-40, IISR-44, IISR-51 and IISR-526, were found promising against *R. solanacearum* *in vitro*.

### Screening of ginger germplasm for bacterial wilt tolerance

Ten short listed germplasm accessions were further screened by soil inoculation method using two different concentrations of *R. solanacearum* and rhizome inoculation method.

None of the accession could survive at a concentration of  $10^8$  where all the plants succumbed to wilt within three weeks of inoculation.

However, at a concentration of  $10^6$  cells there were some surviving plants. When observed for rhizome intactness, all the ginger accessions were found rotting and only the periderm could be seen.

### Detection of *Ralstonia solanacearum* using PCR and ELISA

#### Collection and characterization

Isolates from Ambalavayal, Madakeri, Kothamangalam, Kotappady, Peruvannamuzhi etc. were added to the repository. The

**Table 46.** Changes in the population of *Ralstonia solanacearum* in vascular tissues of ginger rhizomes during rhizome solarization as detected by DAS-ELISA

Time of solarization	Duration of solarization(h)	Rhizome temperature(°C)	A 405 Values*					
			Vadakkanad	Poothady	Kenichira	Beenachy	Ambalavayal	Pulpally
0:00	0	27.55	0.039	0.117	0.020	0.023	0.015	0.440
9:00 to 10:00	1	39.6	0.013	0.007	0.003	0.045	0.213	0.200
9:00 to 11:00	2	50.0	0.009	0.002	0.012	0.031	0.036	0.152
Negative control					0.035			0.282

\* Mean of two replicates, Absorbance was read at 405nm 1 hour after adding the substrate solution

present strength of the collection is 40.

Amplified Ribosomal DNA Restriction Analysis (ARDRA) technique using primers designed from 16 s rDNA and 16-23 s rDNA intergenic regions was standardized for bacterial strain characterization. RFLP using *Msp I* of 16 s rDNA did not produce much polymorphism. However, the technique was found to differentiate the biovars of the bacterium when using restriction enzymes *Msp I* or *Sau 3A-I* to restrict the amplified hyper variable intergenic (16-23S) sequence.

A band with size of 290 bp was found to be specific for Biovar 3 of *R. solanacearum* irrespective of host origin whereas a band with 210 bp was found to be specific for biovar 2 *R. solanacearum* from potato when the amplified intergenic region was restricted with *Sau 3A-I*. The same when restricted with *Msp I* was found to produce a band of size 384 bp specific for biovar 2. A band of 928 bp was found when 16 s rDNA of biovar 2 was amplified which was absent in biovar 3 isolates of different host including ginger.

Table 47. Details of endophytic bacteria isolated from ginger pseudostem

Sl. No	Sample ID	Source	Colony characteristics
1	GEB-1	Healthy pseudostem	Pink, small, irregular, raised, opaque, entire colonies
2	GEB-2	Healthy pseudostem	White irregular, raised, fluidal, entire colonies
3	GEB-3	Healthy pseudostem	Reddish brown, round, raised, opaque, entire colonies
4	GEB-4	Healthy pseudostem	Dark red, circular, raised, opaque, entire colonies
5	GEB-5	Bacterial wilt affected pseudostem	White colonies with red centre, fluidal, irregular, raised colonies.
6	GEB-6	Bacterial wilt affected pseudostem	White colonies with brown centre, fluidal, opaque, irregular colonies
7	GEB-7	Bacterial wilt affected pseudostem	White fluidal colonies with pink centre, irregular, fluidal
8	GEB-8	Bacterial wilt affected pseudostem	Dark red, round colonies with yellow border, fluidal colonies
9	GEB-9	Bacterial wilt affected pseudostem	Dark pink, irregular, raised colonies with white border, fluidal colonies
10	GEB-10	Bacterial wilt affected pseudostem	Dark brown colonies with white border, irregular, flat colonies.
11	GEB-11	Bacterial wilt affected pseudostem	White irregular colonies with red center, fluidal, entire colonies
12	GEB-12	Bacterial wilt affected pseudostem	White, slightly fluidal colonies with brownish red center, seen in rings
13	GEB-13	Bacterial wilt affected pseudostem	Dark red round, raised, entire colonies.
14	GEB-14	Bacterial wilt affected pseudostem	Dark red colonies with orange border, irregular, raised colonies
15	GEB-15	Healthy rhizome	Cream, irregular, raised, fluidal colonies
16	GEB-16	Healthy pseudostem	Pink colonies with white border, circular, raised, fluidal colonies
17	GEB-17	Healthy pseudostem	Brownish red, irregular, flat, opaque colonies
18	GEB-18	Healthy pseudostem	White colonies with pale red center, slightly fluidal colonies
19	GEB-19	Healthy rhizoplane soil	Red, opaque, flat colonies with creamish border
20	GEB-20	Healthy rhizosphere soil	Brownish red, fluidal, raised, opaque, irregular colonies
21	GEB-21	Healthy leaf	Cream, irregular colonies with light pink center, fluidal colonies

### *Survival of R. solanacearum in soil*

Serological assay was conducted to detect *R. solanacearum* on the debris of the rhizomes collected from bacterial wilt affected ginger plants. Only two of 26 samples tested positive for *R. solanacearum* even after enrichment. Healthy rhizomes were planted in the pots where bacterial wilt was previously recorded. Disease could be recorded only after five months in two of the 10 pots. The survival data generated using serological and bioassay method further confirms that the pathogen fail to survive in soil and also in ginger periderm collected from bacterial wilt affected soil.

### *Detection of Ralstonia solanacearum in naturally infected rhizomes*

Mature ginger rhizomes were collected from six different bacterial wilt affected fields of Wyanad (Ambalavayal, Poothady, Pulpally, Kenichira, Vadakkanad and Beenachy). Among the different samples, positive reaction could be noted only from three locations namely, Kenichira, Poothady and Pulpally as their absorbance values were more than three times of that of negative control value.

## 4. Studies on fungal and viral diseases of ginger

(R. Suseela Bhai, A. Ishwara Bhat, K. M. Abdulla Koya and Santhosh J. Eapen)

### *Survey for the incidence and intensity of dry rot and eye rot diseases*

A survey was conducted to study the intensity of these diseases in different ginger growing tracts of Wyanad (9 locations), Calicut (3 locations) and Kannur (3 locations). Samples were collected from spice markets, stored ginger samples and field samples (at the time of harvest from planters) at random. From each location three samples were collected @ of 1 kg per lot and were sorted for dry rot and eye rot diseases. The survey revealed that the intensity of these diseases were below the economic threshold

level. Among the fifteen locations surveyed, eye rot was observed in only three locations and dry rot in two.

### *Studies on etiology and disease development*

A pot culture study was conducted with artificial inoculation of *Pythium myriotulum*, *Macrophomina phaseolina* and *Fusarium oxysporum* individually and in combination. Fungicides such as, carbendazim (0.2%), carbendazim-mancozeb (0.2%), metalaxyl-mancozeb (0.2%) and mancozeb (0.2%) were given individually and along with insecticide dimethoate as differential treatments to control the infection due to these fungi. Fungicides were given as seed treatment and soil application at the time of sowing. The treatments were repeated thrice. First soil application was given at the time of sowing, and further applications at one month interval from the time of germination. From the experiment it was found that *Fusarium* sp. or *Macrophomina* sp. as such were not pathogenic to ginger under our field conditions. Only *Pythium* was found to be highly pathogenic causing soft rot disease. But when the pathogens were inoculated together, there was a decrease in the incidence of soft rot. This may be due to the mutual antagonism existing among the pathogens.

Among the fungicidal treatments, Metalaxyl-Mz was found highly effective in controlling the disease caused by *Pythium* sp. followed by dual application of Metalaxyl-Mz and dimethoate. Application of dimethoate is attributed to the involvement of insect pests in aggravating the disease. As the soft rot disease was aggravated by the presence of insect larvae of *Mimigrella* sp., seed treatment with a systemic insecticide was found useful in reducing the disease incidence. Hence application of dimethoate (0.2%) along with Ridomil-Mz (0.125%) was found promising in controlling soft rot caused by *Pythium* sp.

### *Screening germplasm accessions for soft rot and dry rot disease*

Two hundred germplasm accessions were

screened both against soft rot and dry rot disease. In the case of soft rot, the accessions were rated on a 0-5 scale *ie.* 0 = 0-5% resistant, 1 = 6-20 % moderately tolerant, 3 = 21-50% susceptible, 5 = 51-100%, highly susceptible on the basis of per cent infection of pseudostem. Accessions were short listed based on per cent infection and yield (more than three times the weight of seed. Three accessions namely, Accs. 261, 269 and 274 were found moderately tolerant to soft rot in preliminary screening. This has to be confirmed through secondary screening. 70 accessions were found susceptible to *Macrophomina* infection.

#### *Management of dry rot disease*

Field trial for the evaluation of dry rot disease of ginger with 13 treatments in RBD was conducted in the experimental farm at Peruvannamuzhi. First application of the chemicals were given as seed dressing and soil drenching at the time of sowing and subsequent two applications at one month interval from the time of germination. No disease incidence of any sort could be observed during the crop period. Observations on dry rot disease incidence were taken two weeks after harvest. The disease incidence was found to be less than 1% and all the treatments were at par with each other (Table 48).

The fungus *Macrophomina phaseolina* appears only during the storage period in the rhizomes following the damage of the rhizomes by mechanical means. The fungus is found to be non-infective to the crop under normal field conditions. Therefore soil application of the chemicals or bioagents are not required for reducing the incidence of dry rot disease.

#### *Recording incidence of streak virus*

153 out of 659 germplasm accessions maintained in IISR farm Peruvannamuzhi showed the symptoms of chlorotic leaf streak virus. Rest of the accessions did not show any visible symptoms under natural conditions.

## 5. Investigations on viral and fungal diseases of vanilla

(A. Ishwara Bhat and R. Suseela Bhai)

### *Viral diseases*

A total of 65 vanilla plantations at Idukki, Kozhikode and Wyanad districts of Kerala, and Dakshina Kannada, Udupi and Uttara Kannada Districts of Karnataka were surveyed for the incidence and distribution of viral diseases. Mosaic and necrosis were the two kinds of viral diseases found in many of the plantations. The incidence of mosaic disease varied from 0 to 5% while that of necrosis varied from 0 to 10%. Maximum incidence of mosaic disease was recorded at Adimali under Idukki District of Kerala while many locations surveyed both in Karnataka and Kerala were free from mosaic disease. Similarly, maximum incidence of necrosis disease was observed at Nellipoyil village under Kozhikode taluk of Kerala and many plantations surveyed did not show the presence of necrosis disease. Both the diseases were noticed in all ages of the crop. Arecanut, cashew, gliricidia, erythrina and forest trees were the live standards found in areas surveyed and vanilla was grown under natural shade in these plantations. Both mosaic and necrosis diseases were found in vanilla crop irrespective of the type of standards used. In general both incidence and severity of the disease were higher in Kerala compared to Karnataka.

Disease symptoms were more prominent and visible on the younger leaves (Fig. 15). Various kinds of mosaic and associated symptoms such as mild mottle, mild mosaic, and mild chlorotic mottle streak were observed. In a few cases such mosaic is also associated with leaf distortion giving wavy margin to the leaves. The size of the leaves also gets reduced and in advanced stages leaves become brittle and show severe crinkling with torn areas on the leaf lamina. The necrotic lesion on stem and leaves was the other kind of symptoms found in many



Fig. 15. Virus infected leaves of vanilla

of the plantations. The distinct necrotic lesions of varying length (few mm to several cm) were seen on the stem region of the plant. In a few cases necrosis were also seen in the older leaves at the lower surface of leaves.

Electron microscopy of leaf dip preparations of the diseased plants showed the presence of at least four kinds of particles. Three kinds of flexuous particles of varying length resembling viruses in the genera such *Potyvirus* or *Carlavirus*, *Potyvirus* and *Closterovirus* were observed. These particles were seen independently and also in combination in the diseased samples, thus making it difficult to assign a particular virus for a particular kind of symptoms. In addition, isometric particles were also seen in a few samples in addition to flexuous particles. Studies on the isolation and separation of all the different viruses involved, their purification, identification and characterization are in progress.

#### Fungal diseases

A disease survey was conducted in 46 plantations of Calicut, Idukki and Wyanad Districts of Kerala to study the various disease problems in vanilla. The survey was conducted during the month of April 2003 and August 2003. The main disease noticed during April 2003 was yellowing and immature bean shedding. The disease was

Table 48. Effect of soil application of chemicals and bioagents in controlling dry rot diseases

Treatments	Yield (kg fresh wt.)	Dry rot incidence (%)
Potassium phosphonate (0.3%)	3160	0.06
Carbendazim (0.2%) (Bavistin)	4045	0.12
<i>Trichoderma harzianum</i> (500 g/bed)	4530	1.69
PGPR IISR-51 (48 h old culture in nutrient broth diluted 10 times and applied @ 2 l/bed)	3365	0.56
<i>T. harzianum</i> (500 g/bed)+ PGPR IISR-51	3195	0.75
Phorate (No seed treatment, only soil application @ 100g/bed)	3885	0.51
Carbendazim+ Phorate	3345	0.00
Akomin+ Phorate	3335	0.00
Copperoxy chloride(0.2%)(COC)	4105	0.00
Bordeaux mixture 1%	4700	0.00
COC(0.2% +Phorate)	3845	0.00
BM+ Phorate	4220	0.00
Control (No treatments)	5555	0.72
C.D. (p=0.05)	1275	

also reported from Malappuram and Moovattupuzha areas. The disease appeared from February onwards. The survey revealed the high intensity and wide spread of the disease during the crop season of 2003. The average crop loss estimated due to this disease ranged from 23% to 34%. The disease was characterized by dropping off remnants of flowers from the tip of immature beans which otherwise will remain attached to the beans till half way through maturity. This was followed by yellowing and premature bean shedding. The disease was found to be stress related. The maximum temperature recorded during this period was 35° C and the minimum relative humidity 45%. Laboratory studies conducted with the affected beans revealed the association of *Colletotrichum gloeosporioides*. The fungus was isolated invariably from almost all the samples collected from various localities. High temperature and very low relative humidity prevailed during February to May lead to severe stress which in turn predisposes the plants to infection by *C. gloeosporioides*.





- To develop methods for early diagnosis of virus infection.
- To develop management methods to contain the disease spread and to demonstrate the same in planter's field.
- To identify resistant source.

### *Prevalence of disease*

Surveys conducted in Karnataka indicated that *kokke kandu* was present in 11 taluks, distributed in 5 districts with disease incidence ranging from 0.1% to 99.9%. Nurseries raised in the vicinity of infected plantations also showed the presence of the disease with its incidence ranging from 1.7% to 73.3%. Hongadahalla in Saklespur Taluk (Hassan District) and the entire mixed crop area in Uttara Kannada District were found to be hot spots of the disease. The disease is characterized by clearing of veins, resetting, distinct mottling on leaf sheaths, formation of *hooked tillers and shredding of leaves* during monsoon. Distinct mottling, cracking and deformity of capsules and sterility of seeds are other associated symptoms. Formation of panicles is completely suppressed in severely infected plants.

### *Crop loss*

Studies on yield loss due to the disease carried out under monocropping and mixed cropping systems revealed a loss of 65–99% within 1–3 years of infection.

### *Epidemiology*

The pattern of disease outbreak in different plantations suggested the dual pattern of disease spread. Random spread was noticed in newly planted plantations located 1 to 5 km away from the disease affected plantations. Within the infected plantations both centrifugal and random infections were noticed. A steep disease gradient was observed in the plots with higher percentage of disease incidence. In the selected experimental plots, the disease progress over a period of three years indicated (i) dual pattern of disease spread with solitary

infection in distant blocks/plantation and centrifugal spread around the primary sources of infection, (ii) plotting of disease against distance and duration clearly indicated steep disease gradient followed by shallow gradient in the next 100 m, (iii) initial disease spread pattern suggests that for raising planting material a minimum of 200 m distance from infected plantation should be maintained to avoid chances of infection in the nursery stage, (iv) rate of spread  $r$  in different situations vary from 1.28 to 8.16 per unit/year. The  $r$  dependent on the distance and level of incidence in the surrounding plots/plantations.

Monitoring of aphid *Pentalonia nigronervosa f. caladii* population in different months revealed that aphids exist throughout the year; population starts building up during September and reaches the peak during February to March; population is drastically reduced during monsoon (June–August) and there is no direct correlation between aphid population and rate of disease spread.

### *Transmission and virus - vector relationship*

Transmission studies showed that *kokke kandu* is transmitted by *P. nigronervosa f. caladii*. and there is no seed, soil and mechanical transmission. Studies on mode and pattern of disease spread in mono and mixed cropping systems indicated dual mode of disease spread in plantations. Planting symptomless virus carrying seedlings and clones was the main mode of introduction of the disease into plantations.

Studies with Hongadahalli and Sirsi isolates and respective bio-types of aphid vector *P. nigronervosa f. caladii*, indicated that the virus was acquired within 30 minutes of transmission feeding and persistence of virus in the vector was observed beyond 8 h of acquisition feeding. In serial transmission also the virus persisted beyond 6 h with positive transmission in the 3<sup>rd</sup> transfer. The above studies with different periods of acquisition feeding, transmission feeding, post acquisition

starvation and serial transmission clearly revealed that *kokke kandu* virus can be grouped under semi persistent type.

#### **Mechanical transmission**

Mechanical transmission was tried with borate buffer (pH 8.0) and potassium phosphate buffer (pH 7.5) using combination of antioxidants and abrasive. Totally six hosts namely *Nicotiana tabacum* var. *Harrison special*, *N. tabacum* var. *Samson*, *N. tabacum* var. *Whiteburly*, *N. glutinosa*, *Physalis* sp. and *Elettaria cardamomum* (CCS-1). No mechanical transmission was observed in any of the tested hosts.

#### **Etiology**

##### *EM studies*

Crude and clarified sap, squash preparations from different host parts like leaf, leaf sheath, root and young capsules were observed under EM for detecting associated virus particles. Similarly partially purified and purified preparations obtained after following ten different protocols using four buffer systems suitable for different kinds of viruses were examined under EM. The samples did not reveal the association of any virus.

##### *Serology*

Serological relationship of *kokke kandu* virus was studied by using antiserum prepared against PVY (potato virus Y), HNV (henbane mosaic virus), CABMV (cowpea aphid borne mosaic virus), SCMV (sugarcane mosaic virus), PRSV (papaya ring spot virus), BBTV (banana bunchy top virus), GBNV (groundnut bud necrosis virus), WSMV (watermelon silver mottle virus), TSV (tobacco streak virus) and BGMV (bean golden mosaic virus). No positive reaction was observed with any of the above antisera tried through Direct Antigen Coated-ELISA test.

#### **Management of disease**

The feasibility of roguing as strategy to contain secondary spread of disease was tried in

about 40 ha area representing different field situations like (i) isolated plantations (ii) replanted plantations in infected zone and (iii) replanted plantations lying in contiguous with infected plantation. The cumulative removal of new infections in 1995 varied from 0.28% to 3.28% in different experimental blocks. In three isolated plantations, no fresh incidence was recorded even after two years of roguing the initial infected plants. These results confirmed the previous findings; wherein roguing was indicated as viable strategy in containing the virus spread particularly on community basis in each location. Further, to refine disease management package, many inputs from other aspects of disease management and reducing vector population were tried. This package included training to field staff for correct identification of initial infection; rising of disease free planting material; removal of breeding sites of vector; planting of non-host barriers (coffee, banana, cocoa and pepper) and removal of virus sources through periodical inspections, detection and elimination.

The above integrated package was tested in five plantations located in hot spots of *katte* (mosaic) and *kokke kandu* infections. This approach helped in reducing the spread of both viral diseases to a manageable level (less than 2% infection).

#### **Host resistance**

A large number of natural escapes of vein clearing disease were screened by artificial inoculation and also by exposing them to the disease in sick plots for 3 years. Among these, 72 collections took infection and the remaining 11 collections, which remained symptom less, are under further testing. Screening of 24 elite selections, which included distinct morphotypes, high yielding land races, released and pre-released selections was undertaken in sick plot located in endemic zone. After three years all the collections took infection only one selection [clonal selection 893 (APG-244)] was found

less susceptible to the disease with 33% infection compared to 100% infection in local check.

### Externally Funded Project

1. **DBT: Molecular characterization of viruses causing stunted disease in black pepper and development of PCR based methodology for their detection**

(A. Ishwara Bhat and R. Suseela Bhai)

#### *Amplification of coat protein gene of cucumber mosaic virus*

Total RNAs from cucumber mosaic virus (CMV) infected black pepper plants were extracted using Nucleospin RNA plant Kit (Macherey-Nagel, Duren Germany) were used as a template for reverse transcription (RT). RT-PCR was performed in the same tube without any buffer changes in between. The primer pair designed from the CP gene sequences of cucumber mosaic virus was used in the reaction to prime the amplification. The genome sense primer 5' ATG GACAAA TCT GAA TCA AC 3' derived from the beginning of first 20 bases of coding region and the genome antisense primer 5' TCA AAC TGG GAG CAC CC3 represents the last 17 bases of the CP gene of CMV. The reaction profile involved one cycle of 42 °C for 45 min for cDNA synthesis and 40 cycles of amplification with the following parameters: 30s denaturation at 94 °C, 1 min annealing at 50 °C and 1 min of extension at 72 °C followed by one cycle of final extension for 10 min at 72 °C. RT-PCR was successful in amplifying the CMV coat protein gene from black pepper samples. A PCR product of expected size (ca. 650 bp) was observed only in infected samples and not with healthy black pepper samples.

#### *Cloning*

The PCR product was purified using Strata Prep PCR purification Kit (Stratagene, La Jolla, CA, USA) and the purified products were later on subjected to polishing with *Pfu* polymerase and dNTP mix. The resulted product was then cloned in to pPCR Script

Amp (SK+) Vector using pPCR Script Amp (SK+) Cloning Vector Kit. The *E. coli* cells (DH5 $\mu$ ) were transformed with the ligated product using standard molecular biology procedures. Positive clones were identified by restriction digestion of DNA isolated for each of the clones and also through PCR using CMV CP specific primers.

#### *Sequencing of the clone*

Selected positive clones were sequenced at Avestha Gen Gain Technologies Pvt. Ltd., Bangalore India. The sequenced region contained a single open reading frame of 657 bases that could potentially code for a protein of 218 amino acids.

#### *Sequence comparison*

The coat protein gene of the black pepper isolate was compared with corresponding gene of 26 CMV isolates belonging to both the subgroups from different hosts and region including India at the nucleotide and amino acid levels (Fig. 16). Multiple sequence alignments were made using CLUSTAL W. Sequence phylograms were constructed using PHYLIP package (Bootstrap analysis with 1000 replicates), and rooted trees were generated using TREEVIEW software.

Cluster dendrogram revealed that black pepper isolate was most closely related to members of subgroup I isolates. The coat protein gene of black pepper isolate shared 89 to 95% identity with various isolates of CMV belonging to subgroup I while per cent identity ranged from 75 to 76 with various isolates of CMV in the subgroup II. Similarly, comparison of amino acid sequences of the coat proteins revealed that coat protein of black pepper isolate shared 92–99% identity with members of subgroup I and from 77 to 79% identity with members of subgroup II. An identity of >95% were observed with the available coat protein gene sequences of CMV isolates from India both at nucleotide and amino acid level. So far no sequences of CMV subgroup II is available from India. In general, coat protein amino acid sequence

<u>ATGGACAAATCTGAATCAACCAGTGCCTGGTCGCAACCGTCGACGTCGTC</u>	70
<u>MDKSESTSAGRNRNRNRPRRGRSRS</u>	24
CTTCCTCTCCGGGATGCTACATTTAGAGTCTCTGCGCAACAGCTTTCGGGACTTAATAAGACGTTAGC	140
SSSADATFRVLSQQLSRLNKTLS	47
AGCTGGTCGCTACTATTAACCACCCACCTTTCGGGTAGTAGCGCTGTAACCTGGATACAGGTTTC	210
AGRPITINHPFFVGSERCKPGYTF	70
ACATCTATTACCCTGAAGCCTCCGAAAATAGACAAGGGTCTTATATGGTAAAAGGTTGTTACTTCTG	280
TSITLKPPIDKGSYYGKRLLLLPD	94
ATTCAGTCACTGAGTTCGATAAGAAGCTGTTTCGCGCATTCAAATTCGAGTTAATCCTTCCGGAAT	350
SVTEFDKKLVSRIQIRVNP LPKF	117
TGATTTACTGTGGGTGACAGTCCGTAAGTTCCTGCTCTCGGACTTGTCCGTTTCGCCATCTCT	420
DSTVWVTVR RVPASSDLSVSAIS	140
GCCATGTTTCGGGACGGAGCCTCACCACTACTGGTTTATCAGTATGCCCGTCCGGAGTCCAAGCCAACA	490
AMFADGASPVLVYQYAAASGVQANF	164
ATAAATGTTGTAAGATCTTTCGGGTGATGCGGCTGATATTGGTGACATGAGAAAGTACGCCGTCTCGT	560
KLLIDL SVMRADIGDMRKYAVLV	187
GTATTCAAAGCAGTGCCTCGAGACGGATGAACTAGTACTTTCATGTCGACATTGAGCACCAACGTATT	630
YSKDDALETDELVLLEVVDIEHQRI	210
CCCCTTCTGGGGTCTCCCACTTGA	657
PTSGLVLPV<	218

Fig. 16. Coat protein gene sequence (shown as DNA) and deduced amino acid sequence of cucumber mosaic virus infecting black pepper

When the isolates were subjected to ELISA, majority of the isolates reacted either to Cucumber mosaic virus (CMV) or Banana streak virus (BSV) indicating the involvement of at least two viruses with the stunted disease of black pepper.

CMV isolated from naturally infected black pepper was propagated on *Nicotiana benthamiana* and *N. glutinosa* were used as the source for virus purification.

identity among subgroup I isolates of the same geographic area is much higher than that between isolates of different area. The sequence homology of coat protein gene ranges between 96.3% and 99.5% within a subgroup and between 76.0 and 77.5% for isolates of different subgroups. Homologies in coat protein amino acid sequences are 94.0 to 99.2% and 79.5% to 83.2% respectively. In accordance with this the black pepper isolate belong to subgroup I.

2. ICAR: Identification and development of diagnostics against viruses causing stunted disease in black pepper

(A. Ishwara Bhat and R. Suseela Bhai)

Collection and maintenance of isolates

Sixty five isolates of black pepper representing different geographical areas of Karnataka and Kerala and ten isolates of *Piper longum* showing mosaic mottling symptoms coupled with stunting of entire plants and eight isolates of *P. colubrinum* showing mild mottle symptoms were collected. All the collected isolates are being maintained under insect proof glass house through vegetative propagation.

Virus purification was done using the modified protocol of Lot *et al.* (1972) through differential and sucrose density gradient centrifugation. After sucrose density gradient centrifugation, the virus-containing band was located with the help of light vertically passing through the tube. The band was collected, dialyzed and concentrated by centrifugation at 45,000 g for 3 h. The purification procedure resulted in a clean virus preparation. A single opalescent band was seen in the sucrose density gradient centrifugation. Electron microscopy of negatively stained purified preparations revealed the presence of isometric particles of about 28 nm in diameter.

The antiserum against CMV was produced in New Zealand white rabbit by injecting purified virus preparations intramuscularly six times at 10 day intervals. On each occasion, 500 µl purified virus-containing 0.5 mg of virus emulsified with incomplete Freund's adjuvant (1:1, v/v) was injected. The animal was bled 15 days after the last injection and antiserum collected. Immunoglobulin G (IgG) was purified from the crude polyclonal antiserum by affinity

**Table 50.** Detection of *Cucumber mosaic virus* (CMV) by double antibody sandwich (DAS) ELISA\* in black pepper and other *Piper* species from different regions

Isolate	A <sub>405</sub> value*	Isolate	A <sub>405</sub> value*
<b>Karnataka State</b>			
<i>Hassan District</i>			
Bantana halli	0.43	Sample 4	3.76
Belur	0.57	Sample 5	2.00
<i>Madikeri District</i>			
Balale	0.32	Sample 6	0.76
Kodlepet	0.78	Sample 7	0.71
Mayamudi	0.37	Sample 8	0.57
Polibetta	0.41	Sample 9	0.51
<i>Udupi District</i>			
Idu	0.68	Sample 10	0.35
<b>Kerala State</b>			
<i>Idukki District</i>			
Adimali	0.75	Sample 11	0.33
Chakkuppalam	0.22	Sample 12	0.30
Chottupara	0.19	Sample 13	0.21
Muttom	0.15	<i>P. longum</i>	
Thookupalam	0.19	Sample 1	0.53
Vandiperiyar	0.15	Sample 2	0.46
<i>Wyanad District</i>			
Kenichera	0.49	Sample 3	1.33
Kappadi	0.57	Sample 4	1.65
Pulpally	2.01	Sample 5	1.16
Vengapalli	0.78	Sample 6	0.22
<i>Calicut District</i>			
Peruvannamuzhi	0.61	<i>Nicotiana benthamiana</i>	2.23
<b>Tamil Nadu State</b>			
Walayar	1.16	<i>N. glutinosa</i>	1.98
Healthy black pepper	0.00	Healthy black pepper	0.04
<i>Piper chaba</i>			
Sample 1	0.33	* A <sub>405</sub> value taken one hour after substrate addition	
Sample 2	0.31		
Sample 3	0.3		
Sample 4	0.21		
Sample 5	0.20		
<i>P. colubrinum</i>			
Sample 1	3.92	chromatography. Affinity column contained protein A coupled to cyanogen bromide activated agarose (Genei, Bangalore). 5 ml of polyclonal antiserum was passed through the column and the column was washed with 25 ml of wash buffer to remove all unbound materials. The IgG bound to the column was later eluted by adding 5 ml of elution buffer and quantified by taking OD values at 280 nm (1.4 OD = 1mg/ml of IgG). One mg of this IgG was used for conjugate preparation. One step glutaraldehyde method described by Avrameas (1969) was followed for the preparation of IgG-alkaline phosphatase conjugate.	
Sample 2	3.89		
Sample 3	3.98		
<b>Detection of CMV by double antibody sandwich (DAS) ELISA</b>			
Double antibody sandwich (DAS) ELISA procedure was standardized using CMV IgG			

and conjugate prepared, for the detection of CMV infection in field samples of black pepper. The DAS-ELISA procedure thus standardized detected the CMV in dilutions of extracts from diseased black pepper plants from different regions of Karnataka, Kerala and Tamil Nadu (Table 50). The varying OD values seen with diseased black pepper samples from different regions indicate varying virus concentration in these samples.

#### *Virus elimination through meristem culture*

The shoot tips were collected from the virus-infected black pepper plants (var. Panniyur) grown in the field. These shoot tips as such were surface sterilized and the meristem was dissected out under aseptic conditions. The isolated-meristems were kept in the medium containing MS+1mg/litre IAA for regeneration at 25 °C. Within six days all of them got contaminated and eventually died. Since contamination was the main problem, efforts were made initially to establish plantlets under *in vitro* conditions using shoot tips/nodal segments. A good regeneration medium was established with explants such as shoot tips and nodal segments. From such *in vitro* grown plantlets meristem was dissected out and allowed to regenerate. Because of contamination problem, regeneration could not take place. Different treatments and modification of the media are being attempted to regenerate the plant from meristem, which will enable the production of virus free plants.

#### Final Report

1. ICAR: National network project on *Phytophthora* diseases of horticultural crops

(M. Anandaraj and Y. R. Sarma)

#### *Collection and maintenance of National repository of Phytophthora*

A large number of *Phytophthora* from various host plants have been collected and maintained in the National repository. Presently, 547 isolates infecting 35 host plants have been conserved in the repository.

#### *Characterization of Phytophthora isolates from black pepper*

Growth of black pepper *Phytophthora* isolates on different solid media was evaluated by measuring radial growth of fungus after 48 and 72 h. Maximum growth was on carrot agar (19.5–37.75 mm) followed by papaya dextrose agar (18.0–36.5 mm) and cornmeal agar (16–30 mm). Least growth was on potato dextrose agar (12.0–20.5 mm).

Sporulation of *Phytophthora* was found to be maximum in carrot agar followed by papaya dextrose agar and cornmeal agar and was very less in potato dextrose agar.

#### *pH and growth of Phytophthora isolates from black pepper*

Among the twenty five isolates tested twenty one isolates did not grow at pH 2.5 and all the isolates grew well at pH 5 and pH 7, at pH 9 all isolates showed very less growth.

#### *Morphological characterization of Phytophthora*

A total of 173 isolates of *Phytophthora* from black pepper was characterized morphologically. Among these, 162 isolates were *P. capsici*, three were *P. palmivora*, three *P. parasitica* and another five were atypical isolates. All the *P. capsici* isolates were caducous with long pedicels. Range of pedicel length was 31.4–163.8 mm. *P. palmivora* isolates had pedicel length ranging from 4.3–5 mm and *P. parasitica* with 3.9–6.5 mm. Among 115 isolates of *P. capsici* tested only 60 produced chlamydospores.

#### *Determination of mating type*

All the isolates were tested for mating type by growing the isolates together with known mating types of A1 and A2 in clarified carrot agar supplemented with  $\beta$  sitosterol (30 mg/litre). Out of the one hundred and seventy isolates, one hundred and forty four isolates were A1, six were A2, and twenty isolates were sterile. Among 162 *P. capsici* from black pepper 142 were A1, three A2 and 17 sterile. Out of three *P. palmivora*, two were A2 and

one sterile. Out of three *P. parasitica*, two were A1 and one sterile. The three atypicals were A1 and two were sterile.

### **Pathogenicity**

Pathogenicity of 144 isolates were done by detached leaf inoculation technique on *Piper nigrum*, *P. betle*, *P. longum*, and *P. colubrinum*. They were categorized based on size of the lesion produced.

One hundred and twenty one isolates out of 144 were pathogenic to betel vine leaves and 23 were non pathogenic. One hundred and eighteen isolates were pathogenic to *P. longum* and eighteen isolates produced lesion of 10–19.3 mm size after 72 h on *P. colubrinum* leaves whereas, others did not produce any lesion or produced only hypersensitive reaction.

### **Characterization of *Phytophthora* isolated from betel vine**

Variations among 52 isolates of *Phytophthora* were studied by standard methods. Among the 52 isolates studied 39 isolates were with white fluffy aerial mycelium without any pattern and belonged to A2 mating type, eight were with white cottony mycelium without any pattern, five isolates were with stellate growth pattern and all the isolates produced chlamydospores. The 39 isolates with white fluffy aerial mycelium produced amphigynous antheridia. The rest 13 isolates were A1 mating type and produced amphigynous antheridia. *P. capsici* and *P. parasitica* isolates were responsible for foot rot of betel vine. Among the *P. parasitica*, 16 West Bengal isolates were with fast mycelial growth (39–44.5mm) and L/B ratio was less (1.15–1.3), other 23 *P. parasitica* isolates had comparatively slow growth (26–36.5) and L/B ratio comparatively high (1.3–1.5). Among *P. capsici* there were two types, stellate isolates with fast mycelial growth and L/B ratio high and white cottony slow growers with L/B ratio 2 or less than two. The *P. parasitica* can be grouped into two based on morphology as fast growers with low L/B

ratio and slow growers with high L/B ratio. *P. capsici* can be grouped into two based on morphology as fast growing stellate with L/B ratio of more than 2 and white cottony colony slow growers with L/B ratio of less than 2.

Eighty two isolates from other hosts such as *P. chaba*, vanilla, tapioca, bauhinia, potato, nutmeg, strawberry, citrus, crossandra, gerbera, diffenbachia, papaya etc were morphologically characterized. The species identified were; *P. capsici*, *P. meadii*, *P. palmivora*, *P. parasitica*, *P. boehmeriae* and *P. cinnamomi*

### **Molecular characterization**

UPGMA cluster analysis of isozyme data of black pepper *Phytophthora* grouped the isolates into a major cluster consisting of the typical *P. capsici* isolates and three other clusters. The *P. capsici* isolates had two major sub-groups. The first sub-group consisted of isolates which had an L/B ratio of values equal to or >2.0 and were in general, highly virulent. The second sub-group consisted of isolates with L/B ratios less than or equal to 2.0 and composed of less, moderate and highly virulent types. The second major sub-group consisted of 3 clusters of isolates with a general L/B ratio of values less than or equal to 2.0 and differed from each other based on their virulence. The sterile isolates grouped under the less virulent group. The highly virulent group consisted of floral as well as stellate types. The third cluster consisted of a set of uniform isolates of moderately virulent type with distinct morphological characters. These had a modified chrysanthemum type colony morphology and an L/B ratio of 1.5 and thus formed a unique group among *P. capsici* isolates, possibly forming the *Cap A* group suggested by Mchau and Oudemans based on sporangial dimensions. Nine isolates, morphologically identified as *P. capsici* were off-types, and lacking the typical characters of *P. capsici*, also grouped differently from the major cluster of *P. capsici*.



Among the off-types, *P. palmivora* and *P. parasitica* formed separate groups.

The UPGMA cluster analysis of the isozyme data of betel vine isolates revealed two clusters within *P. parasitica*. The similarity coefficient between the two clusters was > 0.8. Twenty eight electrophoretic types were revealed for 8 loci across four enzyme systems studied for 40 isolates. Out of these, there were three major electrophoretic types, each consisting of 2 or more isolates. 16 *P. parasitica* isolates collected from West Bengal, formed a major electrophoretic type. Each of the other isolates of *P. parasitica* formed a distinct electrophoretic type. This serves as a supportive evidence for their distinct identity based on morphological characters. *P. parasitica* isolate, 98.119 was very distinct from the other isolates for all the enzymes studied and had a coefficient of similarity of only 0.59 with the other isolates. It was noted that there was no correlation between the geographical distribution and the grouping of the isolates among *P. capsici* isolates. However, among *P. parasitica* isolates, there was a distinct separation of the West Bengal isolates from the *P. parasitica* isolates collected from the other regions.

#### ITS -RFLP

Protocols were standardized for isolation of DNA from *Phytophthora* and RAPD and ITS-RFLP. For species identification using ITS-RFLP the genomic DNA was amplified with ITS 4 and ITS 6 and the amplified fragment was restricted with MspI/ AluI and further electrophoresed to give species specific banding pattern.

#### Host pathogen interaction

The mechanism of resistance in the *Phytophthora* tolerant line, P-24 was studied and it was found to be due to early induction of defence enzymes such as phenylalanine ammonia lyase (PAL). There was also induction of pathogenesis related (PR) proteins  $\beta$ -1,3 glucanase and chitinases. There were deposition of auto fluorescing callose

material deposited as the fungus invades the tissues in *Phytophthora* tolerant line

#### Biological control

A total of 585 fungal and 832 *Fluorescent pseudomonads* and *Bacillus* spp. have been isolated and evaluated for their biocontrol activity. The percentage of inhibition varied from 20 to 84%. Most of the *Trichoderma* isolates overgrew *P. capsici* colony within three days. But no parasitism was observed on CA plates. However coiling and penetration of *P. capsici* hyphae by *Trichoderma* was observed on water agar plates. *T. aureoviride* showed such parasitic activity than any other group. From each group promising isolates were selected for *in-vivo* studies.

A total of 56 isolates of bacteria were screened for their antagonism to *P. capsici*. Thirty four isolates were able to inhibit *P. capsici* recording more than 50% inhibition. Glass house experiments were done in 3 sets involving 79 isolates of *Trichoderma*. In the first set, 20 isolates of *T. virens* existing in the repository of IISR were screened. In the first set of bioassay with *T. virens* isolates, four of them namely, IISR-13, IISR-15, IISR-17 and IISR-121 were able to promote plant growth significantly. Isolate IISR-112 was able to suppress *P. capsici*. The disease incidence in that case was 30% as against 90% in the control. In the second set of bioassay with isolates of *T. aureoviride*, *T. hamatum*, *T. harzianum*, *T. polysporum*, *T. longibrachiatum*, *T. koningii* and some isolates of *Trichoderma* spp., 19 of them were identified as plant growth promoters. In the third set of bioassay with isolates of *Trichoderma* spp., six of them namely, IISR-1294, IISR-1306, IISR-1307, IISR-1320, IISR-1321 and IISR-1337 were found to be plant growth promoters. Isolate IISR-1240 and IISR-1325 reduced the disease incidence to 20% as against 80% in the control.

When *T. harzianum* was mixed with neemcake, coir pith and farm yard manure

in 1:20 and 1:40 proportions, the population has increased after 10 days. Then it declined slowly after 30 days. Coir pith and farm yard manure showed maximum number of cfu in the 1:20 and 1:40 proportions.

Twenty isolates of *T. harzianum* were studied for the volatile effects on *P. capsici* isolate 98-162 after exposure for 25 days. *Trichoderma* isolates TH-10 and TH-39 caused greater reduction in the virulence of *P. capsici*. *P. capsici* exposed to *T. harzianum* isolates, TH-21, TH-25, TH-30, TH-45 showed no difference in virulence after one year.

The biocontrol efficiency depended upon the isolate and their population density in soil. In the bioassay using *T. harzianum* P-26, there was reduction in the disease incidence at an antagonist dose as low as  $10^3$  colony forming units per gram of soil whereas, *T. virens* P-12

required  $10^6$  cfu/g of soil for effective suppression of the pathogen. *T. virens* 17 reduced the disease incidence at antagonist dose of  $10^4$  cfu/g of soil. Combination of *T. harzianum* + *T. virens* recorded maximum growth of black pepper. This was followed by single application of *T. harzianum* and *T. aureoviride* which were on par with two-way application of *T. harzianum* + *T. aureoviride*, *T. virens* + *T. aureoviride* and *T. aureoviride* + *T. pseudokoningii*.

#### ***Protoplasts release and regeneration of Trichoderma***

Protocols were standardized for the isolation of protoplasts from *Trichoderma* and their regeneration. Protoplast regenerations were obtained from *T. virens* IISR-1370 and *T. aureoviride* IISR-143.

## VIII. Mega Project: Conventional and molecular approaches for developing pest, pathogen and nematode resistance in spice crops

(Project Leader: M. Anandaraj)

### 1. Screening germplasm of spice crops for reaction to diseases

(R. Suseela Bhai, M. Anandaraj, M. N. Venugopal and K. V. Saji)

#### Screening black pepper germplasm accessions to *Phytophthora rot*

Forty four black pepper cultivars were subjected to preliminary screening with stem inoculation and leaf inoculation. Among them two cultivars (C-1311 and C-1321) were found moderately tolerant. Fourteen short listed hybrids were subjected to secondary screening but none exhibited the tolerance. Screening is progress with OP seedlings raised from 70 accessions including hybrids.

Open pollinated progenies of P-24 and KS-27 were also screened for reaction to *P. capsici*. Among 100 OP progenies of P-24 screened none showed tolerant reaction whereas 4.3% of OP progenies of Subhakara showed tolerant reaction. In P-24 (*Phytophthora* tolerant line) the mechanism of tolerance was found to be due to early induction of defense enzymes such as phenylalanine ammonia lyase (PAL) and induction of pathogenesis related (PR) protein  $\beta$  1-3 glucanases. In the *Phytophthora* tolerant progenies of Subhakara  $\beta$  1-3 glucanase activities were higher compared to parent.

Seedling progenies were raised by selfing Panniyur-1, Subhakara, P-24 and by hybridizing Panniyur-1 and Subhakara. The progenies were screened for their reaction to *P. capsici* and they exhibited varying degrees of resistance to *P. capsici*. Among the progenies 6.0%, 11.1% and 8.0% of Panniyur-1, Subhakara and P-24 respectively did not show lesion at all. The penetration index was also zero for 8.0%, 9.7% and 10.0% of selfed progenies of Panniyur-1, Subhakara and P-24 respectively. In the crossed progeny population of 169 seedlings, 12 seedlings (7.1%) showed resistant reaction and another 26.0% showed tolerant reaction.

### 2. Screening germplasm of spice crops for reaction to nematodes

(Santhosh J. Eapen and K. V. Saji)

#### Black pepper

Forty-five germplasm accessions were screened against *M. incognita* and all the accessions were found susceptible to the nematode. An *in situ* screening experiment was laid out at Peruvannamuzhi Farm to confirm the resistance of nine black pepper germplasm accessions (C-812, C-820, C-1047, C-1090, C-1204, C-4103, HP-39, HP-60 and HP-290).

#### Ginger and turmeric

Fifty each of ginger and turmeric germplasm accessions were screened against *M. incognita*. Out of these, 13 turmeric and 14

**Table 51.** Evaluation of nematode resistant ginger and turmeric germplasm accessions in microplots

Acc. No.	Height (cm)	No. of tillers	Yield (g/plant)	EMI
<i>Ginger</i>				
Acc. 26	81.2ab	21.4b	357c	1.0
Acc. 73	86.9a	19.6b	520ab	0.7
Acc. 202	79.2b	29.1a	528a	1.3
Acc. 217	78.2b	21.4b	497ab	0.7
Acc. 221	71.2c	19.6b	458abc	0.7
Maran (Control)	84.6ab	23.8ab	411bc	0.8
<i>Turmeric</i>				
Acc. 21	98.3b	5.8b	958.0b	0.0
Acc. 82	132.8a	7.3b	1216.0a	1.0
Acc. 84	81.6c	6.4b	1040.0ab	0.7
Prathibha (Control)	129.9a	9.1a	634.0c	2.3

EMI : Egg mass index (0 – 5 scale)

ginger accessions were short-listed for the second round of screening. Nematode resistant accessions namely, Accs. 82 and 84 in turmeric and Acc. 202 in ginger were found superior in yield and other characters under microplot conditions (Table 51).

An analysis of nematode resistance genes was carried out using bioinformatics tools. Based on this, gene-specific primers were designed for *Mi* gene, *APS* gene and others.

### 3. Screening germplasm of spice crops for reaction to insect pests

(K. M. Abdulla Koya, S. Devasahayam, T. K. Jacob, K. V. Saji and B. Sasikumar)

One hundred and sixty five accessions of cultivars and 42 accessions of hybrids of black pepper available in the Germplasm Conservatory maintained at Experimental Farm, Peruvannamuzhi were screened against pollu beetle (*Longitarsus nigripennis*) to isolate sources of resistance against the pest. The pest infestation on berries ranged from 6.2% to 25.5% in the cultivars and from 1.6% to 17.9% in the hybrids.

Five hundred and fifty one accessions of ginger available in the Germplasm Conservatory maintained at Experimental Farm, Peruvannamuzhi were screened against shoot borer (*Conognethes punctiferalis*) to isolate sources of resistance against the pest. The study indicated that the pest infestation ranged from 5.3% to 30.9% in various accessions.

Dried rhizomes of 77 accessions of ginger and

12 accessions of turmeric were screened for damage by cigarette beetle (*Lasioderma serricornis*) in the laboratory. Rhizomes of 2 accessions of ginger were free of infestation.

### 4. Mechanism of resistance to pests and pathogens in spice crops

(M. Anandaraj, B. Chempakam, S. Devasahayam, Santhosh J. Eapen and T. John Zachariah)

#### *Phytophthora* - black pepper pathosystem

RAPD profiles of *Phytophthora* tolerant lines which were identified earlier was done using 15 operon primers along with a susceptible var. Subhakara. One of the primers, OPA-01 showed a distinct band of ~300 bp in all the *Phytophthora* tolerant lines namely, P-24, HP-780, HP-3, HP-1, C-1090 and C-1095. This band was eluted, further amplified by PCR using the same primer and cloned in *Escherichia coli*. The fragment was sequenced in order to develop SCAR marker which could be used for screening large population of seedlings. The fragment contained 373 bp and BLAST alignment showed homology with several resistant genes reported both in plants and other organisms.

#### *M. incognita* - black pepper system

Biochemical analysis of nematode tolerant line of black pepper revealed enhanced activity of defense enzymes such as phenylammonia lyase, catalase, peroxidase and superoxide dismutase. Acid phosphatase showed a decreased activity.

## IX. Mega Project: Developing integrated pest and disease management strategies in spice crops

(Project Leader: S. Devasahayam)

### 1. Disease management in *Phytophthora* foot rot affected black pepper plantations

(M. Anandaraj, V. Srinivasan and C. K. Thankamani)

An experiment to develop a package to rejuvenate foot rot disease affected plantations is in progress with 16 treatment combinations. The components include maintenance of weed cover in the interspaces where zero tillage was followed and in another block clean cultivation was followed by periodical removing of weeds by digging. Other treatments were growing of disease susceptible and tolerant varieties, providing organic and inorganic nutrition and chemical and biological methods of disease control.

The initial growth and establishment of vines was better with clean cultivation and after 3 years the yield was also more in this block. The soil availability of N, P and K were found to be slightly higher in block with weeds as compared to clean cultivation. The available K was higher in plots with inorganic nutrition compared to organic whereas the P availability was higher in organic nutrition plots.

### 2. Utilization of *Piper colubrinum* Link and *P. arboreum* as rootstocks in the management of foot rot disease of black pepper

(P. A. Mathew, J. Rema and T. John Zachariah)

The growth of black pepper grafts in marshy area (IISR farm) on arecanut has been good. In order to achieve good coverage of laterals on the support, planting five grafts on each support was found to be the best instead of single grafts wherein only few laterals were seen on one side of the support. To prevent snapping of graft union due to falling arecanut leaves, grafts were grown close to

the arecanut stem facilitating close tying with support and the grafts were found to grow without any detrimental effect. So far no graft failure or disease incidence has been noticed.

The six year old graft plot in farmer's field at Peruvannamuzhi (Fig. 17) yielded 488 kg of dry pepper from 685 vines. The yield was low compared to last year (735 kg dry). This yield reduction could be due to the drought effect this year which is being ascertained through profuse irrigation. Infection on *P. colubrinum* tissues were noticed in 5 grafts and the samples has been given for pathological investigations.



Fig. 17. Black pepper vines grafted on *Piper colubrinum* rootstock

Due to severe drought during this year grafts in Wyanad, Goa, Kadangode and Thodupuzha perished. One more plot in Thodupuzha has been planted with virus free *P. colubrinum* to be used as rootstock for black pepper.

Quality analysis of berries indicated that the quality of berries was not affected by various methods of grafting in terms of piperine, oleoresin and essential oil.

### 3. Biological control of diseases of spices

(M. Anandaraj, R. Suseela Bhai and A. Kumar)

A new trial on the development of biocontrol consortium for managing *Phytophthora capsici* and plant parasitic nematodes in black pepper has been laid out at IISR farm Peruvannamuzhi. The organisms involved are *T. harzianum* (P-26) and selected bacterial isolates which were short listed from green house studies. The population build up of the introduced organisms and the native populations are monitored along with growth response of black pepper and disease incidence.

Promising isolates of rhizobacteria suppressing both *R. similis* and *M. incognita* were screened against *P. capsici*. IISR-658, IISR-853 and IISR-869 inhibited *P. capsici* in laboratory tests and these were also good P solubilizers. An observational trial on the effect of PGPR has shown that the isolate IISR-6 recommended for black pepper also enhanced the yield of ginger (Table 52). Studies with different isolates of PGPR against soft rot disease of ginger showed that PGPR isolates IISR-13, IISR-51, IISR-151, IISR-152 and IISR-906 were effective in reducing the disease incidence to less than

5%. Endophytic bacteria associated with ginger rhizome and pseudostem was isolated from bacterial wilt affected ginger plants as well as from healthy plants.

Genomic DNA of nematicidal rhizobacteria (from black pepper, cardamom, ginger) was isolated through the CTAB-SDS method and procedure for ARDRA (Amplified Ribosomal DNA Restriction Analysis) finger printing was standardized.

Antibacterial activities of cell free culture filtrate of *T. viride* was found effective against bacterial wilt pathogen, *Ralstonia solanacearum*. The culture filtrate was active against the growth and multiplication of the pathogen *in vitro*. The culture filtrate was inactivated by heat at 70–90 °C. Mutant derivative (albino) of the *T. viride* did not produce antibacterial metabolites.

### 4. Biological control of nematodes of spices (Santhosh J. Eapen and A. Kumar)

#### Studies on PGPR

Promising isolates of rhizobacteria that suppress both *R. similis* and *M. incognita* were screened against *Phytophthora capsici*. IISR-658, IISR-853 and IISR-869 inhibited *P. capsici* in laboratory tests. They are good P solubilizers too (Table 53).

Table 52. Effect of PGPR on growth and yield of ginger and turmeric

Treatment	Ginger		Turmeric	
	No. of tillers	Yield (kg)	No. of tillers	Yield (kg)
IISR-1	297.6a	2.88a	101.0abc	5.46bc
IISR-13	223.2 a	1.25 bc	102.6abc	6.58ab
IISR-51	203.4 abc	1.08 bc	107.4ab	6.86ab
IISR-853	157.6bc	1.44 bc	87.2cd	6.76ab
IISR-859	188.8 bc	1.94 b	104.8abc	7.36a
K-149	149.0 bc	0.78 c	96.4bc	5.52bc
K-151	172.8 bc	1.36 bc	87.6cd	4.66cd
Molasses	228.6ab	1.10 bc	73.4d	3.52d
Control	115.4 bc	0.99 bc	118.2a	4.08cd

Means represented by same letter are not significantly different under DMRT

Table 53. Promising isolates of rhizobacteria that suppressed *Radopholus similis*

Is. No.	Source	Location	Reaction to		P. solubilization	Production of HCN
			Mi	Rs		
658	<i>M. incognita</i>	Wyanad	+	+	+	-
663	<i>P. nigrum</i>	Idukki	+	NT	-	-
853	<i>Chromolaena odorata</i>	Calicut	+	+	+	-
865	<i>Strychnos nuxvomica</i>	Calicut	+	+	+	+

+ Present, - Absent, Mi - *Meloidogyne incognita*, Rs - *Radopholus similis*

### Field trials with promising BCAs

An observation trial was laid out in the Experimental Farm, Peruvannamuzhi to study the management of nematode pests of black pepper by deploying five promising isolates of rhizobacteria (IISR-522, IISR-528, IISR-658, IISR-853 and IISR-865). Among these, IISR-522, IISR-528 and IISR-658 were superior in suppressing plant parasitic nematodes and significantly reducing the foliar yellowing in black pepper vines.

In another ongoing field trial at Peruvannamuzhi using four fungal isolates namely, *Verticillium chlamydosporium*, *T. harzianum*, *Paecilomyces lilacinus* and *Scopulariopsis* sp., *V. chlamydosporium* and *Scopulariopsis* sp. were promising in reducing the foliar yellowing and reducing the

nematode infestation in black pepper.

Two more new trails were laid out at Peruvannamuzhi to evaluate the performance of ginger (var. Himachal) and turmeric (var. Prathibha) when they are supplemented with promising PGPR strains. Ten PGPR strains were evaluated for their efficacy to improve the plant growth and suppress soil borne diseases and nematodes. There were four replications with a plot size of two beds of 3 m x 1 m. The rhizobacteria were multiplied on molasses and applied at the time of sowing (@ 2.5 litre/bed) and after two months. Observations were recorded on the germination of seeds and yield. Incidence of pests and diseases was also monitored. The germination of ginger seeds was significantly improved consequent to the application of

Table 54. Evaluation of promising rhizobacteria for growth and yield of ginger and turmeric

Isolate No.	Ginger		Turmeric	
	Germination (%)	Yield (kg/3m <sup>2</sup> )	Germination (%)	Yield (kg/3m <sup>2</sup> )
IISR-6	76.12a	4.38 b	92.16ab	6.41 ab
IISR-13	78.98a	5.32 a	95.10 a	6.23 ab
IISR-51	77.76a	4.90 ab	85.48 b	6.00 ab
IISR-149	82.54a	4.27 bc	93.79ab	5.92 ab
IISR-151	73.97a	4.27 bc	88.12ab	5.59 ab
IISR-853	84.10a	4.38 b	93.77ab	5.81 ab
IISR-859	73.57a	4.29 bc	87.14ab	6.18 ab
IISR-866	77.04a	4.48 ab	92.62ab	5.82 ab
IISR-522	81.15a	4.12 bcd	91.96ab	4.51 b
IISR-658	77.36a	3.30 d	92.40ab	5.97 ab
Control	59.32b	3.40 cd	93.88ab	6.47 a

Figures followed by the same letter in a column are not significantly different

rhizobacteria. The maximum germination was observed with IISR-853 in both ginger and turmeric. Application of IISR-6, IISR-13, IISR-51, IISR-149, IISR-853 and IISR-866 increased the ginger yield by 33.9%–62.7% (Table 54). However, in turmeric, significant improvement in either germination or yield could not be obtained with any of the bacterial strains.

## 5. Biological control of insect pests of spices

(S. Devasahayam, K. M. Abdulla Koya and T. K. Jacob)

### Management of shoot borer

A commercial neem product (Nimbecidine) and neem oil were evaluated at 1% concentration (that were promising during the previous year) in the field at Peruvannamuzhi for the management of shoot borer (*Conogethes punctiferalis*) on ginger to develop eco-friendly schedules based on organic insecticides for the management of the pest. The neem products were sprayed at 15-day intervals on the experimental plants during July to October. A treatment involving spraying of malathion 0.1% at monthly intervals during July to October was also maintained. However, both the neem formulations were not effective in reducing the pest infestation on the crop and the percentage of shoots infested by the pest was on par with control.

An integrated strategy such as adoption of cultural methods (pruning and destruction of freshly infested shoots at fortnightly intervals during July to August) and spraying of neem products (Neem oil and Nimbecidine - 1% concentration each at 15 day intervals during September to October) were evaluated in the field at Calicut for the management of shoot borer on ginger. Treatments involving pruning and destruction of freshly infested shoots at 15 day intervals during July to August and spraying of malathion 0.1% at monthly intervals during September to

October and spraying of malathion 0.1% at monthly intervals during July to October were also maintained. However, the treatments involving pruning of infested shoots and spraying of neem products were not effective in reducing the pest damage on ginger.

### Management of rhizome scale

Dried leaves of four plant species namely, *Chromolaena odorata*, *Glycosmis pentaphylla*, *Melia composita* and *Strychnos nux-vomica* were evaluated as storage material for the management of rhizome scale (*Aspidiella hartii*) on ginger rhizomes during storage. The rhizomes were dipped in quinalphos 0.075% and stored in various plant products. The trials indicated that, storage of rhizomes in dried leaves of *S. nux-vomica* and *G. pentaphylla* after dipping in quinalphos 0.075% were promising for obtaining a higher recovery of rhizomes, higher number of sprouts and lesser incidence of rhizome scale.

Another trial involving evaluation of promising storage materials such as dried leaves of *S. nux-vomica* and *G. pentaphylla* along with saw dust in various proportions for the management of rhizome scale is under progress.

## 6. Characterization of bioactive compounds with pesticide properties

(N. K. Leela, M. Anandaraj, S. Devasahayam and Santhosh J. Eapen)

### Characterization of fungitoxic compounds

In earlier studies two compounds were isolated from the fungitoxic fraction of *C. odorata* leaves of which one compound was identified as 2'-hydroxy-3, 4, 3', 4', 6'-pentamethoxychalcone by spectral studies.

### Isolation of active principles in *Annona squamosa* seeds

In order to isolate the nematocidal compound, the methanol extract of *Annona squamosa* seeds, which showed nematocidal activity



against *M. incognita*, was divided into ethyl acetate soluble and insoluble fractions. These fractions were further separately fractionated by column chromatography into 19 groups, 13 from ethyl acetate soluble and 6 from ethyl acetate insoluble material. Preliminary screening of 6 major fractions indicated that two out of these fractions caused 39% and 32% nematicidal activity at 0.25% concentration. Similarly antifeedant activity of the major fraction from hexane extract was also tested against pollu beetle. This fraction (hexane-ethyl acetate 98:2) at 2% concentration caused 92% feeding deterrence against pollu beetle.

### Final Report

#### 1. Bioecology and management of mealybugs infesting black pepper

(K. M. Abdulla Koya, S. Devasahayam and M. Anandaraj)

#### Objectives

The project aims at studying the incidence, nature of damage, life history and developing management schedules against mealybugs infesting black pepper.

#### Distribution

Surveys conducted in Wyanad District during 1998 revealed that 20–80% of the vines were infested by mealybug *Planococcus* sp. during monsoon period. The incidence was found to be 4.4–17.8% during summer months. During 1999, the incidence varied from 6.7% to 42.2% when the survey was carried out in nine locations. However, at two locations mealybugs were not noticed. Surveys conducted in Calicut District during 2000 showed that mealybug infestation was negligible in these areas except in a few gardens in Chakkittapara area. During 2001, surveys conducted in north eastern part of Calicut District indicated that mealybug infestation could not be located in 12 gardens visited. Out of 39 gardens surveyed spread over 13 locations in Kodagu District of Karnataka, 3 gardens showed medium and 13 gardens mild infestation by *Planococcus* sp.

### Nature of damage

Studies conducted on nature of damage caused by mealybugs indicated that they were present on main roots as well as on secondary and tertiary roots and were found to be present even up to a depth of 2 feet below the soil on the roots. Many of the vines having mealybug infestation were also affected by *Phytophthora* foot rot disease and such vines exhibited yellowing, defoliation and wilting.

### Life history

The morphometrics of adults and crawlers were determined. The total life span from crawler to adult ranged from 32 to 36 days. The total number of crawlers produced by female ranged from 19 to 197.

### Standardization of techniques for mass culturing

Seven host materials such as elephant foot yam, colacasia, coleus, potato, pumpkin and squash were evaluated for culturing mealybugs under laboratory conditions. Pumpkins and squash were most ideal for mass culturing.

### Management of mealybugs

Preliminary field trails using neem products and organic compounds revealed that neem products did not have any impact on the mealybugs. However, chlorpyrifos at 0.1% was most effective when the root zone of black pepper vines were drenched with the insecticide solution. A field trail was conducted at Kalpetta using insecticides such as quinalphos, malathion, chlorpyrifos and prophenophos all at 0.075% concentrations singly and in combination with wetting agent for management of the mealybug. The insecticide application was carried out during May and August and observations on incidence of root mealybug were carried out 30 days after the final treatment (drenching with insecticides).

Combined analysis of data of two years showed that all the insecticides were effective

in reducing the population of root mealybug when compared to control. However, wetting agent alone was found to be equal to untreated control. Among the various treatments, chlorpyrifos was the most effective and was on par with quinalphos and prophenophos (Table 55).

Table 55. Efficacy of insecticides against root mealybug

Treatment	Population/2.5 cm of root
Quinalphos 0.075%	0.5 de
Chlorpyrifos 0.075%	0.0 e
Prophenophos 0.075%	1.6 cd
Malathion 0.075%	2.2 c
Quinalphos 0.075% + Sandovit 0.1%	1.1 cde
Chlorpyrifos 0.075% + Sandovit 0.1%	0.0 e
Prophenophos 0.075% + Sandovit 0.1%	1.1 cde
Malathion 0.075% + Sandovit 0.1%	2.5 c
Sandovit 0.1%	7.7 b
Control	9.4 a

(DMRT test at  $P < 0.05$ )

### Externally Funded Projects

#### 1. IFS : Development of disinfection technology for management of bacterial wilt of ginger

(A. Kumar)

Disinfection of rhizomes with solar radiation, a method called rhizome solarization, is being developed in the present project for bacterial wilt management with the help of grants obtained from IFS (International Foundation for Science, Sweden). The basic information on the effect of heat on viability and pathogenic ability of *R. solanacearum* and the effect of heat on germination, viability and emergence of ginger (rhizomes) was studied in the project. The survival of the bacterium on ginger rhizome before, during and after heat treatment through rhizome solarization was studied using serological approaches. Technology demonstration trails were conducted in green house as well as in farmer's field. The series of experiments conducted in the project are furnished below.

#### Effect of heat on survival of *R. solanacearum*

At a temperature of 46 °C complete killing was observed when exposed for 1 h. As the temperature increased, the survival of bacterium decreased. When the cultures were exposed for 30 min at 46 °C, 98.0% killing could be recorded, whereas 100 per cent death could be observed upon longer (60 min) exposure. Thus the thermal inactivation point of *R. solanacearum* was determined to be 47 °C at 30 min. The experiment was repeated as described above with narrow temperature range (45.6–46.5 i.e., 45.6, 45.7, 45.8, 45.9, 46.0, 46.1, 46.2, 46.3, and 46.4 °C in order to know the thermal death point of *Ralstonia* at 30 minutes of exposure. Complete death of all the cells could be seen at a temperature of 45.6 to 46.4 °C.

#### Effect of heat on pathogenicity of *R. solanacearum*

When the heat-exposed cells (40 and 45 °C) were inoculated, the expression of the wilting symptom was delayed for 2 days compared to check (30 °C), where the wilt could be noticed in 6 days. When the exposure was above 45 °C, the pathogen could not wilt the plants due to complete death of all the cells at this temperature.

#### Rhizome solarization

When the seed rhizomes were exposed for 28 days (8 to 224 h) of day light, the sprouts got burnt and the rhizomes turned soft and rotten. The burnt appearance of the sprout could be due to high temperature (> 50 °C). The extended period of rhizome solarization has detrimental effect on rhizomes and germination. Even at 8 h of solarization, the sprouts were completely burnt and the rhizomes were rotten after a week of storage. Prolonged exposure to heat has been shown to be detrimental to germination of ginger.

Larger rhizomes recorded 1–3 °C higher temperatures than the smaller rhizomes. As the size increased the heat build up also increased. The variation in the heat build up

in the rhizome could be due to the fact that the larger seed rhizome has a larger surface area to trap the sunlight, which in turn results in higher temperature in rhizome. Relationship between rhizome size and heat buildup has also been recorded.

Wilt incidence was recorded after 45 days of planting and 35% of the plants emerged from unsolarized seed pieces expressed wilting symptoms after 60 days, the incidence was 75% on 90<sup>th</sup> day and none of the plants survived after 100 days of planting. Plants emerging from solarized rhizomes often escape the disease due to *in situ* killing of the pathogen in the seed rhizome or in the vascular tissue itself.

#### *Serological approaches*

Serological evidence for elimination of *R. solanacearum* from ginger rhizomes was recorded. Negative results obtained in post enrichment DAS-ELISA for *R. solanacearum* in solarized rhizomes confirm that the bacterium is not surviving in solarized rhizomes. The assay clearly indicates that rhizome solarization is capable of disinfecting the rhizomes infected by *R. solanacearum* either artificially or naturally. The temperature generated inside the rhizome has decreased the number of viable bacteria in the rhizome

Rhizome washings and vascular extract obtained from solarized rhizomes were subjected to DAS-ELISA in order to know the fate of *R. solanacearum*. The data on the A405 values indicates that the solarization has got detrimental effect on *R. solanacearum* in vascular tissues as extract from 1 and 2 h failed to give positive colour reactions (0.512 and 0.80 respectively) whereas the extract from the unexposed rhizome has tested positive for *R. solanacearum* (1.450).

Rhizomes collected from ginger plants emerged from solarized infected rhizomes tested negative for *R. solanacearum* in post enrichment NCM-ELISA.

#### *Green house and field trials*

Field trials were conducted in five locations for two years in the state of Kerala and Tamil Nadu namely, Kothamangalam, Kottapady, Kannur, Ambalavayal, Pulpally, Gudalur, and Peruvannamuzhi for two years using the ginger rhizome (local cultivar) available with the farmers. Rhizomes were solarized for different duration (0, 30, 60, 90, 120 min. from 9 to 11 am) in the forenoon. 20 kg of rhizome was solarized for specified duration and planted in 20 beds @ 1 kg per bed immediately after solarization. Post enrichment DAS-ELISA was used to detect the inoculum of *R. solanacearum* in unsolarized and solarized rhizome as well as in soil before planting. The data on heat buildup in rhizome, heat retention in rhizomes, status of rhizomes, germination, disease incidence and yield was collected and is being compiled.

#### 2. BRNS: Migration of pesticides in soil, water and plant environment – A study using <sup>32</sup>P labeled potassium phosphonate

(R. Suseela Bhai and K. Vasu)

#### *Translocation studies*

Plants were raised from runner vines as well as from laterals (bush pepper) for translocation studies. These plants would be inoculated with p<sup>32</sup> labeled and unlabeled potassium phosphonate. Plants treated with potassium phosphonate would be inoculated with *Phytophthora capsici* at different time intervals to study the persistence of potassium phosphonate inside the plant system. The experiment is in progress.

#### 3. DBT: Endophytic bacteria for biological system management of *Radopholus similis*, the key nematode pest of black pepper (*Piper nigrum* L.)

(K. V. Ramana, Santhosh J. Eapen, R. Ramakrishnan Nair and A. Kumar)

One hundred and three strains of endophytic bacteria were isolated from various plant parts of black pepper and rhizosphere soil through different approaches. Bacteria seen

along with the tissue cultured black pepper plants were also isolated. They were cryopreserved at  $-80^{\circ}\text{C}$  in 20% glycerol and were maintained for further assays. These isolates were characterized by colony morphology, antibiotic sensitivity, their ability to colonize black pepper plants and nematicidal properties. For testing their nematicidal activity, they were suspended in sterile distilled water and were serially diluted up to  $10^{-6}$ . The whole assay was performed in 24-well microtitre plates. After 72 h, the number of live and dead nematodes was counted under a stereomicroscope by adding a few drops of 1N NaOH. Among the 25 isolates screened, the nematicidal activity ranged from 0% to 29% under *in vitro* conditions. For molecular characterization of the efficient strains, ARDRA was standardized. Analysis for developing primers specific to biocontrol genes has been completed. A novel method based on staining with Fluorescent DiAcetate (FDA) to differentiate live and dead nematodes was developed.

#### 4. ICAR: Bioecology and integrated management of root mealy bug (*Planococcus* sp.) infesting black pepper

(S. Devasahayam, K. M. Abdulla Koya and M. Anandaraj)

##### *Distribution of root mealybug*

Surveys were conducted in Idukki (15 locations), Kannur (9 locations) and Kasaragod (9 locations) districts of Kerala and Dakshina Kannada (4 locations), Udupi (1 location), Uttara Kannada (4 locations) and Hassan (9 locations) districts of Karnataka to record the incidence of root mealybug infesting black pepper in these areas. Seven gardens in Idukki District and five gardens in Hassan District were infested by the pest.

##### *Mealybug species infesting roots of black pepper*

Collections of root mealybug infesting black pepper were made from the various locations surveyed and preserved in the laboratory for identification. Two species of *Planococcus* sp. were found to be associated with roots of

black pepper. The correct identity of the species is being determined.

##### *Nature of damage*

The nature of damage caused by root mealybug infesting black pepper was studied. Colonies of root mealybug were distributed on the main, secondary and tertiary roots on rooted cuttings in the nursery and also on vines of all age groups in the field. The basal portions of the stems of the vines were also infested when they were under soil/leaf mulch. The pest infestation resulted in defoliation, yellowing and wilting of leaves and lateral branches. Experiments to study the interaction of root mealybug and other pathogens such as *Phytophthora capsici* and nematodes observed in infested vines were set up under green house and simulated field conditions.

##### *Bioecology*

Methods were standardized for studying the biology of root mealybug in the laboratory. Studies on life history of root mealybug are in progress. Studies on morphometrics and duration of various stages is under progress. The seasonal incidence of root mealybug in the field is being monitored at Wyanad.

The alternate hosts of root mealybug were recorded in black pepper gardens infested with root mealybug. Colonies of root mealybugs were observed on banana rhizomes and base of stems of coffee and *Erythrina* sp. and also on roots/base of stems of 11 weed plants (belonging to the families Amaranthaceae, Asteraceae, Compositae, Euphorbiaceae, Fabaceae, Graminae, Malvaceae and Oleandraceae). The correct identity of the mealybugs are being determined.

##### *Mass culturing*

Nine fruits/vegetables/tubers belonging to Aroidae, Cucurbitaceae, Zingiberaceae, Rutaceae and Solanaceae, namely, colocasia, elephant foot yam, pumpkin, squash, ash gourd, bottle gourd, cucumber, water melon, citrus, ginger, turmeric and potato are being evaluated for their suitability for mass culturing of root mealybug.

## Final Report

1. DBT: Compatibility, stability and potential of biocontrol consortium on suppression of *Phytophthora* foot rot of black pepper and their conservation

(Y. R. Sarma, M. Anandaraj and A. Kumar)

Fluorescent pseudomonads and *Trichoderma* spp., were isolated from black pepper roots and rhizosphere soil collected from different places in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Sikkim. A repository of rhizobacteria is maintained at IISR, Calicut with a present strength of 905 strains. The antagonistic potential of bacterial isolates and *Trichoderma* spp. were evaluated by dual culture technique against *P. capsici*, the foot rot pathogen of black pepper.

Mode of action of PGPRs in protecting black pepper from infection by *P. capsici* was studied in detail. Strains of fluorescent pseudomonads, IISR-13 and IISR-51 significantly inhibited the radial growth of *P. capsici*. Strains also produced inhibitory HCN, siderophores and antibiotics namely, pyoluteorin and pyrrolnitrin. The efficient strains of *Pseudomonas fluorescens* namely, IISR-6, IISR-8, IISR-11, IISR-13 and IISR-51 and *Trichoderma* spp. namely, isolates, P-12, P-26, GV-19, Tav-25 and Th-39 were found to produce mycolytic enzymes namely,  $\beta$ -1,3 glucanases,  $\beta$ -1,4 glucanases and lipases. The efficient strains were characterized based on its efficiency in utilization of different carbon sources, antibiotic sensitivity, and utilization of succinic acid. The efficient bacteria were also found to solubilize phosphate and thus making it available for the plant. The introduced biocontrol bacteria in black pepper was found to be endophytic as revealed by fluorescent microscopic observations.

The selected isolates of PGPR were evaluated in green house for their efficiency in growth promotion, biomass production and foot rot suppression in black pepper. The *P. fluorescens* strains IISR-8, IISR-11 and IISR-51 were effective in protecting black pepper from its three pathogens namely, *R. similis*, *M. incognita* and *P. capsici*.

Studies on the rejuvenative capacity of fluorescent pseudomonads in black pepper indicated the potential of these strains for the nursery management of black pepper, especially to protect the plants from *P. capsici* infection. The shelf life of *Trichoderma* in different coir pith based substrates were tested and was found that coir compost + sorghum is the best carrier media. The shelf life was found to be 75 days. In order to produce bulk inoculum, locally available agricultural waste namely, coconut water and molasses (0.5%) were exploited for multiplication of the bacterial biocontrol agents and was found to be a economical carrier media. In 32 h, molasses supported a population size of  $10^{13}$  per ml and coconut water supported  $10^{15}$  per ml.

With a view to developing a biocontrol consortium in a multiple cropping system involving several spice crops, the efficient biocontrol agents in black pepper were tested against the pathogens of ginger and cardamom and found that disease suppression extends even to these crops, apart from promoting the growth in these crops. The combination of *T. harzianum* isolate, IISR-1369 and *P. fluorescens* strain, IISR-11 could improve the vigour of the plant both in black pepper and ginger. The same treatment combination imparted maximum yield in ginger and cardamom.

In field trials conducted with different combination of fluorescent pseudomonads and *Trichoderma*, the best treatment was found to be a combination of *Trichoderma* spp. (Is. no. IISR-143 and IISR-369) and *P. fluorescens* (IISR-6) to decrease the root rot disease besides increasing the yield.

The two efficient biocontrol agents namely, *P. fluorescens* (IISR-6) and *T. harzianum* (P-26) had been proved individually efficient in protecting black pepper from root rot caused by *P. capsici* and are compatible with each other for the formulation of a microbial consortium to get greater protection and these strains were found to be rhizosphere competent.

## X. Mega Project: Economics, statistics and modeling

(Project Leader: M. S. Madan)

## 1. Economics of spices production and marketing

(M. S. Madan)

*Estimation of cost of cultivation of spices*

Surveys were conducted in vanilla growing districts of Karnataka and Kerala to estimate the cost of cultivation of vanilla. There was a rapid increase in area under vanilla cultivation in the states of Kerala and Karnataka (Table 56). Low price for other competing crops and prevailing better prices for vanilla were the major reasons for increased area expansion. The estimated cost of cultivation indicated the Benefit Cost Ratio of 3.3 and 3.8 in Karnataka and Kerala respectively. The estimated maintenance cost was Rs. 21,084 acre/year. The incurred cost towards compost formed the major component (66%) followed by labour charges (21%). While estimating the cost of cultivation, the value of the land was not taken into account. On an average the total cost of production per acre (700 vines) in first year of bearing was Rs. 27,542. The average yield per acre was 82.25 kg of green beans and the average gross returns realized per acre by selling green beans was Rs. 90,475. The average net return per acre was Rs. 62,933 with the Benefit Cost Ratio of 3.28 (Table 56).

Economics of on-farm processing (curing) of vanilla indicated that, sale of processed vanilla beans returned a huge net benefit of Rs. 54,825/q over the sale of green beans (Tables 57 and 58). Large farms performed better than small farms in vanilla cultivation mainly because of their on-farm processing activities. In the absence of established marketing system, the growers depend on few processors and exporters for onfarm processing and marketing of their produce.

Table 56. Cost of cultivation of vanilla

Particulars	Cost (Rs./acre)
<i>Establishment cost per acre (700 vines)</i>	
Labour	10050.00
Planting material	4560.00
Manures	28000.00
Chemicals	1548.00
Standards for support	2280.00
Total establishment cost	46438.00
<i>Maintenance cost per acre (700 vines)</i>	
Labour usage for all operations	4350.00
Compost	14000.00
Chemicals	645.00
Interest on working capital @11% per annum	2089.45
Total maintenance cost	21084.45
<i>Output and returns per acre (700 vines):</i>	
Apportioned investment cost @ 11%	6458.00
Total maintenance cost	21084.45
Total cost of production	27542.45
<i>Returns</i>	
Average annual production, kg/acre (green)	82.25
Gross returns @ Rs.1100/kg	90475.00
Net returns	62932.55
Cost of production/kg	334.86
Net returns/kg	765.13
Benefit Cost Ratio	3.28

*Economic evaluation of technology*

Economics of using variable rate (low input) of fertilizer and application of biocontrol measures in pepper (Table 59) and organic cultivation of pepper was also worked out using the agronomic data from farmers field from four districts of Kerala (Kasaragod, Kannur, Calicut and Wyanad).

## 2. Identification of appropriate prediction systems in spice crops

(K. N. Kurup and P. Rajeev)

Analysis of impact of establishment of WTO on export and export price of Indian spices are given in Table 60.

Table 57. Processing cost per quintal of vanilla beans

Materials and labour	Quantity	Unit cost (Rs.)	Total value (Rs.)	Cost (Rs./q)
<i>Washing beans</i>				
Women labour	2	50	100	100
<i>Killing process</i>				
Container	1	800	800	136
Thermometer	1	30	30	30
Jute bags	10	15	150	150
Men labour	2	60	120	120
Women labour	1	50	50	50
<i>Sweating</i>				
Wooden box	6	300	1800	487
Blanket	25	150	3750	2190
Women labour	18	50	900	900
<i>Slow drying</i>				
Stand	1	3000	3000	812
Men labour	1	60	60	60
<i>Grading and packing</i>				
Polythene self-stickable bags	20	2	40	40
Women labour	2	50	100	100
Total				5,175

Note: The life of blankets two years ; The life of wooden box stand five years ; Investment on blankets, wooden box and stand is amortized at 11% over the life span of each material.

Table 58. Cost and returns from on-farm processing of vanilla beans

Particulars	Total value (Rs.)
Selling of 1 q green beans @ Rs.1100/kg	1,10,000.00
Processing cost/q of green beans	5175.00
Selling 20 kg processed beans (1:5 ratio) @ Rs.8500/kg	1,70,000.00
Deduction of processing cost from returns of dry sale	164825.00
Net extra income by processing/q of green beans	54825.00

### 3. Remote sensing and GIS in evaluating the impact on socio-ecological changes on spices production in Western Ghats region

(M. S. Madan, V. Srinivasan, K. Kandiannan, K. V. Saji and Utpala Parthasarathy)

Through rapid rural survey and bench mark survey basic parameters to be included in the study were identified in the target district

(Wyanad). Soil map for the state of Kerala including the target district were procured from National Bureau of Soil Survey and Land Use Planning, Bangalore and digitized using GIS software. Watershed maps of the target district were collected and is being digitized. Socio-economic (census) data pertaining to the district were also collected.

Table 59. Economics of low input usage in pepper production

Treatment No	Mean return		Risk table		Marginal return	
	Value of output (Rs./ha)	Cost of input (Rs./ha)	Mean Net Benefit (Rs./ha)	Index variability	Marginal net benefit	Marginal rate of return
T-1(Check)	11908.10	0	11908.10	49.33	-	-
T-2	13876.70	5428.39	8208.31	76.63	-	-
T-3	13085.40	5449.28	7396.12	90.35	-	-
T-4	15412.10	5427.40	9744.70	71.19	-	-
T-5	14803.10	5427.18	9135.92	85.81	-	-
T-6	14282.00	5448.80	8595.20	94.22	66.18	(2) 337.3
T-7	15497.90	5447.02	9810.88	88.66	6.02	(1) 1584.3
T-8	15504.30	5447.40	9816.90	80.00		

T2: FYM 10 kg + 220 g urea + 220 g rock phosphate + 235 g potash/vine ; T3: FYM 10 kg + 220 g rock phosphate + 235 g potash + 1.7 kg neem cake /vine ; T4: FYM 10 kg + 110 g urea + 110 g rock phosphate + 235 g potash/vine ; T5: FYM 10 kg + 235 g potash/vine , T6: FYM 10 kg + 110 g rock phosphate + 235 g potash + 20 g biofertilizers/vine , T7: FYM 10 kg + 110 g urea + 220 g rock phosphate + 235 g potash + 20 g biofertilizers/vine ; T8: FYM 10 kg + 110 g urea + 110 rock phosphate + 235 g potash + 20 g biofertilizers/vine

### Externally Funded Project

#### 1. NATP: Integrated National Agricultural Resources Information System

(M. S. Madan, V. Srinivasan and K. S. Krishnamurthy)

A 'Spice database' with two components of database namely crop details and crop

statistics were developed and put to use. The 'Spice database' is linked to the Data warehouse which was developed at Indian Agricultural Statistics Research Institute, New Delhi. The database softwares were populated with collected secondary data and photos for varietal identification and disease/pest symptoms. Efforts were made to collect recent data on spices production from different state government departments for updating the information.

An interactive expert system for vanilla was developed and a digital code book for spice crops was developed. The expert system for black pepper ('Black pepper anthology') was updated.

Table 60. Impact analysis on export price of spices

Item	Change in export (qty)	Change in export (price per ton)
Pepper	6663 t (+)	Rs. 16370 (-)
Ginger	15612 (+)	Rs. 11306 (+)
Turmeric	2552 (-)	Rs. 6278 (+)



## XI. Mega Project: Extension and training

(Project Leader: P. Rajeev)

### 1. Training of research and extension workers

(P. Rajeev and T. K. Jacob)

The institute offered training programmes on demand from various agencies for field extension functionaries of line departments and research workers of other ICAR institutes and state agricultural universities. The modules for these training programmes are prepared based on the technologies developed by the institute. The topics covered included spices production technology, nursery management in spices, pest and disease management in major spices, post harvest technology and computer applications in which 82 trainees participated. A training on 'Spices research and development' was offered to post graduate students in which 12 students participated.

### Externally Funded Project

#### 1. KRPLLD: Integrated disease management in black pepper - A study on technology diffusion and impact

(P. Rajeev)

Data collected from a sample of 50 planters in Wyanad District of Kerala using a pre tested interview schedule to study the level of adoption, cost benefit and constraints in adoption of integrated disease management technology for the control of foot rot disease of black pepper, include review of literature, stratified random sampling, preparation and pretesting of data collection tools and data collection through sample survey. The data collected is being tabulated and subjected to analysis using appropriate statistical tools.

### 2. NATP : Agricultural Technology Information Centre

(P. Rajeev)

An income of Rs. 1, 48,786/- was generated through sale of planting material, *Trichoderma* sp., publications, CD roms and through diagnostic services. farmers (1572) and students (529) visited the centre for availing various services from the institute.

Three extension seminars were held during the period and two exhibitions were organized in connection with the Kerala Science Congress and AICRPS annual workshop during January and February 2004, respectively.

A touch screens facility was installed at the ATIC. The subject matter domain on "Production technology of ginger" and "Black pepper anthology" was provided based on which software was installed in the touch screen.

### 3. DBT : Distributed Information Subcentre

(Santhosh J. Eapen)

During 2003-04 the centre organized two major events. The centre took the initiative to bring together scientists of agriculture research sector to discuss about various issues on bioinformatics. A two-day workshop on "Agricultural Bioinformatics" was held during 29-30 October 2003. About 50 delegates from various research organizations and agricultural universities participated in it. A 21 days training programme on "Bioinformatics and Biotechnology - Tools and Applications" was organized during 2-23 December 2003. The databases and software developed by the centre include i) *Phytophthora* information resource (PIR)-a comprehensive web resource on *Phytophthora* species ii) a database of

*Curcuma* species, iii) a database of *Myristica* species and Nutmeg germplasm, iv) a database of chemical constituents of cardamom oil and their metabolic pathways, v) Phytfinder-an expert system for identification of *Phytophthora* species based on their morphological characters and vi) an expert system for biovar characterization of *Ralstonia solanacearum*.

The infrastructure was further strengthened through adding more hardware components and several bioinformatics software packages. About 15 more reference books on bioinformatics were added to the collection during the reporting period. The centre has collaborated with DOEACC Centre, Calicut to organize Bioinformatics O and A level courses during 2003. The centre also distributed the DBT studentship and traineeship to eligible candidates.

Besides, the centre also undertook routine services like computational and information support to various R&D projects of the institute, maintenance and updating of the institute website ([www.iisr.org](http://www.iisr.org)), designing and developing databases and software related to spice bioinformatics, sustaining the intranet 'SPICENET' and the e-mail facility 'Spicemail', E- journals etc. and providing round the clock access to internet, bioinformatics tools etc. through the facility called E-Lab.

#### 4. NATP: Prioritization, Monitoring and Evaluation

(K. V. Ramana and M. S. Madan)

##### *Impact of operation and management process*

Six NATP projects and equal number of non-NATP projects were selected for the study. The research team working under the projects were interviewed and the comparative performance of research projects under NATP and non-NATP streams on utilization of funds; impact of operation and management reforms; human resource development and integration of operation

and management processes were analysed. In case of NATP projects, the research facilities in terms of computer, photocopier etc. was augmented than non-NATP projects. A better emphasis on multi-institutional projects has been observed under NATP projects when compared to non-NATP projects. The culture of operating NATP projects is slowly influencing the other institute projects and their function. The PME activity has influenced to a great extent in influencing the research management activities of the institute.

With the provision of HRD in research project under NATP, the mobility of scientists within the country has increased. Scientists have been able to interact more with their peers, which helped in technology design and dissemination. Research productivity in terms of research papers has been found better in case of non-NATP projects as compared to NATP projects during the last three years. The reason would be that as NATP project were started only recently, the time required for the research output to appear in the form of publications in journals may be longer.

##### *Analysis of research impact*

Two NATP projects of the institute were selected for the purpose and were analysed for their research impact using the recommended tools. The result of the research impact study include economic analysis of technologies developed under the project. Economics of implementing soil and water conservation measures under cardamom and coffee based cropping system in Coorg District of Karnataka was worked out. The recommended technology of protective irrigation to cardamom and coffee in summer months using sprinkler system provides an extra benefit of Rs. 73208/ha. with an investment of Rs.12, 292 per annum extra, the realized extra yield reported was more than 50% than that in non-adopter's farm (Table 61).

Table 61. Sprinkler irrigation: Cost-benefit details in cardamom\*

<i>Cost (Rs/ha)</i>	
Labour requirement	3900.00
Amortized capital investment @11 %	7592.00
Running cost	800.00
<b>Total</b>	<b>12,292.00</b>
<i>Returns (Rs/ha)</i>	
Irrigated cardamom (396 kg)**	178200
Non-irrigated cardamom (206 kg)**	92700
Net benefit over control	85500
Annual expenditure towards irrigation (protective)	12,292
<b>Net returns</b>	<b>73,208</b>

\* Number of rounds of irrigations-16.00

\*\*Price of cardamom Rs. 450/kg

The income from vegetative barriers like pineapple, french bean and ginger was also worked out. With no extra expenditure involved either for planting or its maintenance the reported yield of pineapple was nearly 200 kg/acre with an annual income of Rs. 2000. As an extra income the farmer gets around 81 kg of french bean cultivated as hedge crop in the cardamom field. Ginger can be cultivated only in the first year of the cardamom crop. Cultivation in subsequent years is not possible because of disease problem and shade. The average yield estimated was 1029 kg/acre.

# Technology Assessed and Transferred

One black pepper and two turmeric varieties were recommended for release during this year. The details of the varieties are detailed below.

## Black pepper

1. Variety	IISR - Shakthi (P-24)
Year of release	Proposed for release in 2004
Pedigree	Selection from open pollinated progenies of Perambramundi
Areas of adaptation	All pepper growing areas
Average yield	5755 kg/ha
<i>Quality characters</i>	
Piperine	3.3%
Oleoresin	10.2%
Essential oil	3.7%
Dry recovery	43%
<i>Special characteristics</i>	a. Tolerant to <i>Phytophthora</i> foot rot disease b. High dry recovery

## Turmeric

1. Variety	IISR Alleppey Supreme
Year of release	Proposed for release in 2004
Pedigree	Clonal selection of Alleppey Finger Turmeric (AFT)
Areas of adaptation	Kerala, Maharashtra, coastal Karnataka and North Bengal
Crop duration	210 days
Average yield	5.58 t/ha (dry)
<i>Quality characters</i>	
Curcumin	5.5%
Oleoresin	16.0%
<i>Special characteristics</i>	Resistant to leaf blotch

2. Variety	IISR Kedaram
Year of release	Proposed for release in 2004
Pedigree	Germplasm selection
Areas of adaptation	Kerala, Maharashtra, Coastal Karnataka and North Bengal
Crop duration	210 days
Average yield	5.28 t/ha (dry)
<i>Quality characters</i>	
Curcumin	5.7%
Oleoresin	14.0%
<i>Special characteristics</i>	Resistant to leaf blotch

# Education and Training

Post graduate studies

## Ph. D

Geetha S. P. *In vitro* technology for genetic conservation of some genera of *Zingiberaceae*. University of Calicut, Calicut.

Leela N. K. Studies on the isolation and characterization of flavones and triterpenoids from a few plants. Cochin University of Science and Technology, Cochin.

Narayana Kurup K. Marine fisheries and its impact on the economic development of fishermen of Kerala State. Agricultural Economics, Kanpur University, Kanpur.

Prathapan K. D. Systematic studies on flea beetles of South India (Coleoptera: Chrysomelidae: Alticinae). Bangalore University, Bangalore.

Santhosh J. Eapen. Biological control of plant parasitic nematodes of spices. University of Calicut, Calicut.

Sushama Devi C. K. A quantitative analysis of Indian research output on spices since 1970-2002. Annamalai University, Tamil Nadu.

## M. Sc projects

Forty students from various universities undertook their M. Sc project work in Biotechnology, Bioinformatics, Biochemistry, Chemistry, Horticulture, Microbiology, Plant Pathology and Economics under the guidance of the scientists of the institute.

## Training programmes attended by staff

Management Development Programme for Women Scientists, National Academy for Agricultural Research Management, Hyderabad, 27 May-7 June 2003 (R. Suseela Bhai, N. K. Leela).

Statistical Software for Data Analysis, National Academy for Agricultural Research Management, Hyderabad, 18-28 June 2003 (K. N. Shiva).

Summer School on Application of Remote Sensing and GIS in Agricultural Statistics, Indian Agricultural Statistics Research Institute, New Delhi, 6-26 August 2003 (S. J. Ankegowda).

The Cyberary: A Platform for Information Management and Networking, MANAGE, Hyderabad, 11-14 August 2003 (Santhosh J. Eapen).

Agricultural Research Prioritization Techniques, National Academy for Agricultural Research Management, Hyderabad, 21-27 August 2003 (S. Devasahayam, J. Rema).

Advances in Videography and Photography, National Academy of Agricultural Research and Management, Hyderabad, 26 August-5 September 2003 (K. M. Prakash, A. Sudhakaran).

Revised Accounting Procedure at ICAR, National Institute of Financial Management, Faridabad, 1-9 September 2003 (M. K. Sachidanandan, V. L. Jacob).

Introduction to GIS and its Applications, National Remote Sensing Agency, Hyderabad, 1-26 September 2003 (M. S. Madan, K. S. Krishnamurthy, V. Srinivasan).

Winter School on Spatial and Non-spatial Databases for Agricultural Research System, Indian Statistical Research Institute, New Delhi, 9-29 September 2003 (K. Kandiannan).

Computer Applications for Administrative and Financial Management, National Academy of Agricultural Research and Manage-

ment, Hyderabad, 16–23 September 2003 (P. V. Sali, C. Ramesh Babu).

Recent Techniques in Plant Genetic Engineering and Molecular Breeding, National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, 25 September–15 October 2003 (K. V. Saji).

Training on HPLC: Analysis of Vanillin, Capsaicin, Aflatoxin and Sudan-1 Dye, Spices Board, Cochin, 13–17 October 2003 (N. K. Leela).

Protection of Intellectual Property and Agricultural Research, National Academy of Agricultural Research and Management, Hyderabad, 14–17 October 2003 (E. Jayashree).

Physical and Chemical Parameters of Spices, Spices Board, Cochin, 27–31 October 2003 (E. Jayashree).

Techniques in Biochemistry and Molecular Biology, Indian Agricultural Research Institute, New Delhi, 1–21 November 2003 (R. Suscela Bhai).

Winter School on Recent Advances in Survey Sampling with Emphasis on Computer Intensive Data Analysis Techniques, Indian Agricultural Statistics Research Institute, 5–25 November 2003 (P. Rajeev).

Refresher Course on Biological Control of Crop Pests, Project Directorate on Biological Control, Bangalore, 10–15 November 2003 (T. K. Jacob).

Course on Bioinformatics, Indian Institute of Technology, Mumbai, 17–22 November 2003 (Santhosh J. Eapen).

Recent Advances in Organic Farming Technologies in Plantation Crops, Central Plantation Crops Research Institute, Kasaragod, 18–27 November 2003 (S. Hamza).

Recent Techniques in Plant Genetic Engineering and Molecular Breeding, National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, 28 November–18 December 2003 (K. S. Krishnamurthy).

Recent Techniques in Gene Cloning and Functional Genomics, Central Institute of Medicinal and Aromatic Plants, Lucknow, 1–10 December 2003 (A. Ishwara Bhat).

Biotechnology and Bioinformatics, Tools and Applications, Indian Institute of Spices Research, Calicut, 2–23 December 2003 (T. John Zachariah).

Impact Analysis of Agricultural Research and Development, National Academy of Agricultural Research and Management, Hyderabad, 4–10 December 2003 (T. K. Jacob).

XIX National Training Programme in Electron Microscopy for Scientific Investigators, All India Institute of Medical Sciences, New Delhi, 2–21 February 2004 (R. Ramakrishnan Nair).

Hi-tech Floriculture, Indian Institute of Horticultural Research, Bangalore, 3–10 March 2004 (P. S. Manoj).

Training Course on Biotechnology and IPR, National Law School of India University, Bangalore, 17–22 March 2004 (Santhosh J. Eapen).

#### Training programmes organized by the institute

Training Programme on Financial and Administrative Procedures, 23–24 April 2003.



Fig. 18 A training programme in progress

Summer Training on Biotechnology/Biochemistry and Bioinformatics, 5 May–4 June 2003 (Fig.18).

Biotechnology of Black pepper, 1 June–31 August 2003.

Spices Production Technology, 11 June 2003, 19–21 January 2004, March 2004

Refresher Training Programme for Technical Assistants of IISR, 6–7 August 2003.

Experimental Design and Computer Applications in Spices Research, 16–26 September 2003.

Biotechnology and Bioinformatics, Tools and Applications, 2–23 December 2003.

Viral Disease and Nursery Management in Black pepper, 14 January 2004.

#### National Informatics Centre on Spices

The library of the institute under the National Informatics Centre on Spices was set up to



Fig. 19 National Informatics Centre on Spices

provide support to research activities of the institute and to function as a national information storage, retrieval and dissemination system for spices and related areas (Fig. 19).

At present, the library has a collection of 3952 books, 2621 bound volumes, 2271 reprints, 808 technical reports and 110 theses. The library is subscribing to 34 foreign journals and 64 Indian journals in addition to CABCDs and AGRISCDs. The new additions added to the library during the year include 152 books, 30 reprints, 53 technical reports, 5 theses, 50 project reports and 5 CD-ROMs.

The library provides bibliographic services (published in the Journal of Spices and Aromatic Crops) and database services and publishes 'Agri-science Tit Bits' at quarterly intervals. Sharing of resources between the libraries of Central Plantation Crops Research Institute, Kasaragod and IISR, Calicut, was initiated and duplicate subscription of costly journals was avoided. The content pages of journals were scanned and hosted on the institute web site and research articles from journals were sent as pdf files through e-mail to users.

The library has automated a majority of its operations using the library management software LIBSYS. Bar-coding of the entire stock was done using Datamax printer. Two SOUL softwares were procured one for KVK, Peruvannamuzhi and another for Regional Station, Appangala. The National Agricultural Technology Project allotted a sum of Rs. 5 lakhs for subscribing foreign journals.



## *Awards and Recognitions*

Diby Paul. Young Scientist Award 2004, for the best oral presentation for the research paper "Mechanism of suppression of *Phytophthora* root rot of black pepper (*Piper nigrum* L.) by strains of *Pseudomonas fluorescens*" at the XVI Kerala Science Congress, Calicut, 29–31 January 2004.

S. Hamza. S. N. Ranade Memorial Award 2003 for Excellence in Micronutrient Research, for the Ph.D thesis "Zinc and molybdenum nutrition of black pepper (*Piper nigrum* L.) for yield and quality".

T. K. Jacob. State Level Commendation Certificate 2002–03 by National Bank for Agricultural and Rural Development, for his outstanding contributions to KVK in the field of VVV club activities.

K. Kandiannan. Foreign Examiners Dona-

tion Prize for the best student in Agronomy and Prof. S. Subramanian and Scholars Award 2003 for the best thesis "Influence of varieties, time of planting, spacing and nitrogen levels on growth, yield and quality, crop-weather and growth simulation modelling and yield forecast in turmeric", Tamil Nadu Agricultural University, Coimbatore.

IISR Awards of Excellence 2002–03 were presented to C. Ramesh Babu for the best administrative staff; V. P. Sankaran for the best technical staff; Balakrishnan Nair and B. M. Sheshappa for the best supporting staffs.

V. A. Parthasarathy. Member, International Society for Horticultural Science, Belgium.

V. A. Parthasarathy. Technical Expert, Indo-Vietnam Work Plan 2002–03, Vietnam, 2–9 November 2003.

# *Linkages and Collaboration*

## **Agency**

## **Linkage**

National Bureau of Plant Genetic Resources,  
New Delhi.

Research collaboration in collection and conservation of germplasm.

Central Tuber Crops Research Institute,  
Thiruvananthapuram.

Research collaboration in post harvest technology

Centre for Water Resources Development  
and Management, Calicut.

Research collaboration in translocation studies of fungicides.

Rajiv Gandhi Centre for Biotechnology,  
Thiruvananthapuram.

Research collaboration in molecular markers.

Kerala Agricultural University, Trissur.

Research collaboration in biotechnological approaches for improvement of spices and evaluation of tissue cultured plants; Centre for Post Graduate studies.

Indian Agricultural Research Institute,  
New Delhi

Electron microscopic studies.

University of Calicut, Calicut.

Centre for Post Graduate studies.

Bharathiar University, Coimbatore

Centre for Post Graduate studies.

Nagarjuna University, Nagarjunasagar

Centre for Post Graduate studies; MOU for teaching and training M. Sc Biotechnology students.

Department of Electronic Accreditation of  
Computer Courses, Calicut

Teaching and training in bioinformatics

Spices Board, Kochi.

Training programmes.

Directorate of Arecanut and Spices  
Development, Calicut.

Planting material production; training programmes

Department of Agriculture/Horticulture  
of States.

Transfer of technology; training programmes.

# All India Coordinated Research Project on Spices

The All India Coordinated Research Project on Spices (AICRPS) is the largest network in the country to conduct and coordinate the spices research in 19 coordinating and 8 voluntary centres.

## Crop improvement

The AICRPS centers strengthened the genetic resources of spice crops and the germplasm was evaluated for different parameters and the promising accessions in each crop were identified. At present, the germplasm holdings of AICRPS centers consist of black pepper (650), cardamom (369), ginger (644), turmeric (1307), tree spices (228) and seed spices (3901).

At Ambalavayal, black pepper accessions, Panchami, Panniyur-2, Panniyur-3, Panniyur-4, Acc. 2426 and Acc. 2445 were found promising for the high range region of Kerala. Cardamom accessions, CL-692, CL-730 and D-237 were found promising and are included in the varietal evaluation trials at Mudigere.

In ginger, highest fresh rhizome yield was recorded in V<sub>3</sub>S<sub>1</sub>-8 (28.25 t ha<sup>-1</sup>) at Pottangi. Besides, ginger accessions with desirable quality parameters were identified. The dry matter content of ginger accessions varied from 13.00% to 22.50%. The oleoresin and essential oil content ranged between 4.00% to 9.67% and 0.25% to 2.00%, respectively and crude fibre varied from 3.93% to 5.95%. In turmeric, highest fresh rhizome yield was recorded in PTS-39 (26.62 t ha<sup>-1</sup>) under initial evaluation trail at Pottangi. At Solan, five collections namely, ST-365, BDJR-1244, CIs-29, PTSS-24 and DKH-26 showed increase in yield to the extent of 17.64% to 32.62% over the check.

For successful hybridization in cumin, emasculation should be done before 10 am in slightly pink and unopened flower bud and pollination on the next day or third day or second and third day (twice) after emasculation between 11 am to 7 pm. Fenugreek accessions, HM-444 (23.9 q ha<sup>-1</sup>), a green seed coat mutant and HM-372 and HM-376, yellow seed coat mutants gave highest seed yield (33.85 and 32.65 q ha<sup>-1</sup>, respectively). Among them, HM-444, is also resistant to both downy mildew and powdery mildew diseases. At Jobner, RTP-4 proved its superior performance by yielding 1518.33 kg ha<sup>-1</sup> followed by RTP-8 (1477.67 kg ha<sup>-1</sup>) and RTP-9 (1471.33 kg ha<sup>-1</sup>), which will be evaluated under CVT. Besides, several varieties/lines were identified for yield and quality attributes through CVT/CYT.

## Crop management

Twenty one projects are being operated in 11 programmes in crop management to identify good agronomic practices (GAP). In black pepper-arecanut mixed cropping system, irrigation with 20 litre vine<sup>-1</sup>day<sup>-1</sup> and a fertilizer dose of NPK 100:40:140 g vine<sup>-1</sup> recorded the highest yield (2.69 kg vine<sup>-1</sup>) at Sirsi. In a drip irrigation trial in black pepper at Panniyur, irrigation @ 2 litre day<sup>-1</sup> increased the yield (1.74 kg vine<sup>-1</sup>) and among the varieties, Panniyur-5 responded positively and recorded the highest yield (2.08 kg vine<sup>-1</sup>).

Biofertilizers are being recommended to substitute inorganic fertilizers and to improve the quality of the produce in spice crops. Inorganic N 75% + *Azospirillum* 50 g + FYM 10 kg recorded the highest yield of 6.41 kg vine<sup>-1</sup> in black pepper at Sirsi. In an organic farming experiment on black pepper,

application of FYM 10 kg + burnt earth 10 kg vine<sup>-1</sup> has given the highest yield at Sirsi. In cardamom also, highest yield (490.8 g plant<sup>-1</sup>) was recorded with the application of *Azospirillum* 50 g + FYM 10 kg.

Similarly higher yield (22.03 t ha<sup>-1</sup>) in ginger was obtained by applying inorganic nitrogen in combination with *Azospirillum* and FYM. Highest yield of fresh rhizome in turmeric was recorded with the application of N 100% + *Azospirillum* 50 g + FYM 5 kg at Pottangi, Kumarganj and Raigarh centres, while at Coimbatore centre it was with inorganic N 50% + *Azospirillum* 5 kg ha<sup>-1</sup>.

In coriander, application of inorganic N 100% + *Azospirillum* + FYM 5 t ha<sup>-1</sup> recorded maximum seed yield by Jobner centre, while it was with the application of inorganic N 50% + *Azospirillum* 1.5 kg ha<sup>-1</sup> at Coimbatore. However, Kumarganj obtained maximum seed yield by applying FYM 5 kg + *Azospirillum* 50 g. At Jobner, maximum seed yield of 3.20 q ha<sup>-1</sup> was recorded in cumint with the application of N 100% + *Azospirillum* + FYM 5 t ha<sup>-1</sup>.

Maximum seed yield of fennel was recorded with the application of N 100% + *Azospirillum* + FYM 5 t ha<sup>-1</sup> at Jobner, while at Kumarganj centre with FYM 10 t ha<sup>-1</sup> + *Azospirillum* 50 g. In fenugreek, application of inorganic N 100% + *Azospirillum* 1.5 kg ha<sup>-1</sup> as seed treatment + FYM 5 t ha<sup>-1</sup> produced the highest yield of 732 kg ha<sup>-1</sup> at Coimbatore, whereas at Kumarganj, FYM 10 kg + *Azospirillum* 50 g gave maximum seed yield of 18.76 kg ha<sup>-1</sup>.

The role of micronutrients in increasing the crop production was established in ginger. Two sprays of ferrous sulphate 1% at 45 and 55 days after sowing and soil application of zinc sulphate 10.0 kg ha<sup>-1</sup> increased the yield in ginger at Dholi. In coriander also, maximum seed yield of 20.03 q ha<sup>-1</sup> was obtained with soil application of copper sulphate 12.5 kg ha<sup>-1</sup> and 0.25% foliar spray at Kumarganj.

At Dapoli, October to March was found to be the most congenial period for softwood

grafting in nutmeg, particularly January with a success of 68.33%.

### Crop Protection

Black pepper cuttings planted in solarized soil significantly increased sprouting, number of roots and length of roots. Further, solarized soil fortified with *Trichoderma harzianum* and VAM recorded minimum incidence of the *Phytophthora* disease with maximum sprouting. Metalaxyl Gold MZ and *T. harzianum* combination was found to be highly effective in controlling the foot rot disease of black pepper at Pampadumpara and Sirsi centres.

Population of root grubs was reduced significantly with the application of carbofuran (100 g plant<sup>-1</sup>) followed by imidacloprid 0.5 ml l<sup>-1</sup> and increased the yield in cardamom.

The loss due to fungal diseases was low (13.0%) in rhizomes treated with SAAF + *T. harzianum* in the storage with highest recovery (84.5%) of healthy rhizomes. Rhizomes treated with hot water at 51 °C for 10 min and *T. harzianum* mixed with neem cake resulted in minimum incidence of rhizome rot disease with maximum yield at Kumarganj.

Foliar diseases like leaf spot and leaf blotch in turmeric could be effectively controlled by application of mancozeb + carbendazim as seed treatment and foliar spray. At Jagtial, rhizome rot of turmeric was reduced by 37.86% by application of FYM with *T. viride* + *P. fluorescens* @ 12.5 kg ha<sup>-1</sup> as basal and 25.0 kg ha<sup>-1</sup> as top dressing followed by application of FYM with *T. viride* + *P. fluorescens* to seed @ 4 g kg<sup>-1</sup> of seed. However, at Coimbatore application of NPK 125:60:90 kg ha<sup>-1</sup> + FYM 10 t ha<sup>-1</sup> + *T. viride* + *P. fluorescens* @ 4 g kg<sup>-1</sup> as seed treatment + *T. viride* + *P. fluorescens* applied to soil @ 12.5 kg ha<sup>-1</sup> as basal and 25.0 kg ha<sup>-1</sup> as top dressing, respectively recorded the lowest incidence of rhizome rot with highest yield.

In coriander, *T. harzianum* when used both as seed treatment and soil application re-

duced wilt incidence significantly and increased the yield at Jobner, while at Coimbatore, three sprays with neem seed kernel extract 5% was found effective in reducing the disease and increasing the yield. *T. harzianum* with and without carbendazim and neem cake as soil application reduced the incidence of wilt in cumin at Jobner. Lowest incidence of wilt (15.0%) with grain yield of 229 g plot<sup>-1</sup> was recorded in plots where *T. harzianum* was applied through seed treatment + soil application along with mancozeb 0.3% spray. Soil and seed treatment with *T.*

*harzianum* was also found significantly effective for both wilt and blight diseases at Jagudan. For the control of aphids in cumin, two sprays of monocrotophos and acephate were found more effective. Carbendazim as seed treatment alone or along with soil drenching proved highly effective against root rot in fenugreek. Soil application of *T. viride* (5 kg ha<sup>-1</sup>) + neem cake (150 kg ha<sup>-1</sup>) also reduced the disease incidence at Coimbatore.

Besides, several germplasm accessions/varieties resistant to important pests and diseases in spice crops were identified.

# Krishi Vigyan Kendra

## Training programmes

Krishi Vigyan Kendra (KVK) has conducted 91 training programmes on various subjects during the year in which 1754 persons participated (Table 62). An advanced training programme of one month duration was organized for 20 educated unemployed rural women in bamboo handicrafts to equip them for self employment.

## Front line demonstrations

### *Production of organic cowpea*

Two demonstrations were conducted on production of organic vegetable cowpea var. Sharika and Arka Garima in an area of 0.4 ha each (Fig. 20).



Fig. 20. Demonstration on production of organic cow pea

## *Cultivation of okra*

Cultivation of a high yielding mosaic resistant variety of okra var. Arka Anamika was demonstrated in 10 locations in an area of 0.4 ha.

## *Rearing of Kuttanad layer ducks*

Two demonstrations were conducted in Changaroth and Peruvannamuzhi villages on accessing the production performance of Kuttanad layer ducks var. Chara and Chemballi under backyard system of rearing. Observations are in progress on performance of the ducks on egg laying.

## On-farm trials

### *Management of fruit fly in bittergourd*

Three trials in an area of 0.2 ha each on management of fruit fly in bittergourd (var. Preethi) is in progress. Pheromone traps were set in the 2<sup>nd</sup> week of March 2004 and a large no. of flies are being collected in the trap.

### *Growing of grafted pepper in swampy areas*

Two trials on utilization of grafted pepper in swampy areas using the varieties of black pepper released from IISR and Karimunda is in progress in an area of 0.3 ha. Even though all the grafts established well in the beginning the unexpected long spell of drought has resulted in the depletion of soil

Table 62. Training programmes conducted during the year 2003-04

Category	No. of courses	No. of participants			No. of SC/ST participants
		Male	Female	Total	
Practising farmers	78	717	817	1534	140
Rural youth	11	105	94	199	16
Extension functionaries	2	6	15	21	-
Total	91	828	926	1754	156

moisture status killing about 80% of the plant population.

#### *Effectiveness of prostaglandin in cattle*

A trial on studying the effectiveness of prostaglandin  $F_2 \alpha$  in anoestrus dairy cattle is in progress. No pregnancy has been confirmed till date.

#### **Demonstration units**

Demonstration units on improved varieties of black pepper, medicinal plants, mushroom spawn production, model homestead garden, model arecanut seed garden, cashew and nutmeg scion bank, guava, sapota, vanilla and poultry were maintained.

#### **Plant and Animal Health Centre**

KVK also operates a Plant and Animal Health Centre offering various services to the farmers (Fig. 21). An artificial insemination facil-



Fig. 21. Animal clinic at KVK

ity is available at the centre to upgrade the genetic stock of livestock. The centre offers consultation, treatment and doorstep services with a nominal fee. In addition, the centre also provides vaccination facility and organises animal health camps in association with the state animal husbandry department. The centre undertook 475 consultancy/advisory/home services, 311 artificial inseminations, 145 vaccinations of poultry birds and animals and organized 15 animal health campaign/infertility camps during the year.

#### **Revolving Fund**

KVK has a strong revolving fund programme to generate income for productive uses. Under this programme, quality planting materials of allspice seedlings, nutmeg graft, garcinia graft, mango graft, guava layer, arecanut seedlings were produced and sold to public at affordable rates. The veterinary wing of KVK also fetched moderate income to the revolving fund by way of consultation and doorstep services.

During the period an amount of Rs. 4, 06,832 lakh was earned through sale of planting materials, mushroom spawn, *Trichoderma* and Rs. 22,395 through the activities of Plant and Animal Health Centre.

#### **Kisan mela and exhibitions**

KVK regularly participates in exhibitions and seminars within and outside the district. KVK has participated in two exhibitions/Kisan melas during the period.

# Publications

## Books and chapters in books

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## Research articles

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# On-going Projects

## Institute Projects

### **I. Mega Project: Collection, conservation, characterization and cataloguing of germplasm of spice crops for yield and other economically important characters**

1. Gen. I (813): Collection, conservation, cataloguing and evaluation of black pepper germplasm
2. Gen. IX (813): Collection, conservation, cataloguing and evaluation of cardamom germplasm
3. Gen. II (813): Collection, conservation, cataloguing and evaluation of germplasm of ginger and turmeric
4. Gen. VI (813): Collection, conservation, cataloguing and evaluation of germplasm of tree spices
5. Gen. XIII (813): Collection, conservation and improvement of vanilla
6. Hort. III (813): Development of paprika for warm humid tropics

### **II. Mega project: Breeding improved varieties of spice crops for yield, quality, drought and resistance to pests and diseases**

1. Gen. VII.1 (813): Breeding black pepper for high yield, quality, drought and resistance to pests
2. Biotech. IV (813): Biotechnological approaches for crop improvement in black pepper
3. Biotech. VI (813): Development of DNA markers for marker assisted selection in black pepper
4. Gen. X (813): Breeding cardamom for high yield and resistance to *katte* disease

5. Hort. IV (813): Rootstock-scion interactions in tree spices

### **III. Mega project: System approach for sustainable production of spices**

1. SSc. II (813): Nutritional requirement of improved varieties of spices
2. Agr. XXI (813): Efficacy of biofertilizers on nutritional management of black pepper
3. Agr. XXII (813): Biometeorological investigations and modeling in black pepper
4. Agr. XXIII (813): Development and evaluation of soil and water conservation measures and land use systems for sustainable crop production in Western Ghats of coastal region

### **IV. Mega project: Production physiology of spice crops**

1. Biochem. I (813): Biogenesis of pigments in spice crops
2. Phy. V (813): Characterization of drought tolerance in black pepper
3. Phy. VI (813): Characterization of drought tolerance in cardamom
4. Phy. VII (813): Physiological and biochemical basis for productivity in black pepper

### **V. Mega project: Value addition and post harvest processing of spices**

1. PHT. I (813): Quality evaluation in spices

### **VI. Mega project: Production of nucleus planting materials of improved varieties of spice crops**

1. Agr. XX (813): Production of nucleus planting materials of improved varieties of spice crops

**VII. Mega project: Identification, characterization and development of diagnostics against pests, pathogens and nematodes of spice crops**

1. Path. XII (813): Investigations on stunted and phyllody diseases of black pepper
2. Path. XIII (813): Investigations on spike shedding of black pepper at high altitudes
3. Path. XI (813): Studies on bacterial wilt of ginger
4. Path. XIV (813): Studies on fungal and viral diseases of ginger
5. Path. XV (813): Investigations on viral and fungal diseases of vanilla
6. Nema. III (813): Investigations on nematodes associated with spices

**VIII. Mega project: Conventional and molecular approaches for developing pest, pathogen and nematode resistance in spice crops**

1. Crop Prot. I.1 (813): Screening black pepper germplasm for reaction to diseases
2. Crop Prot. 1.3 (813): Screening black pepper germplasm for reaction to nematodes
3. Crop Prot. 1.2 (813): Screening black pepper germplasm for reaction to insect pests
4. Crop. Prot. II (813): Mechanism of resistance to pests and pathogens in spice crops

**IX. Mega project: Developing integrated pest and disease management strategies in spice crops**

1. Path. II.3 (813): Disease management in *Phytophthora* foot rot affected black pepper plantations
2. Hort. II (813): Utilization of *Piper colubrinum* Link and *P. arboreum* as rootstocks in the management of foot rot disease of black pepper
3. Biocontrol I.1 (813): Biological control of diseases of spices

4. Biocontrol I.3 (813): Biological control of nematodes of spices
5. Biocontrol I.2 (813): Biological control of insect pests of spices
6. Org. Chem. II (813): Characterization of bioactive compounds with pesticide properties

**X. Mega project: Economics, statistics and modeling**

1. Econ. I (813): Economics of spices production and marketing
2. Econ. II (813): Identification of appropriate prediction systems in spice crops
3. Econ. III (813): Remote sensing and GIS in evaluating the impact on socio-ecological changes on spices production in Western Ghats region

**XI. Mega project: Extension and training**

1. Ext. IV (813): Training of research and extension workers

**Externally aided projects**

**ICAR Cess Fund**

1. Organization of ginger and turmeric germplasm based on molecular characterization
2. Bioecology and integrated management of root mealy bug (*Planococcus* sp.) infesting black pepper
3. Identification and development of diagnostics for the viruses causing stunted disease in black pepper

**Department of Biotechnology**

1. Conservation of spices genetic resources in *in vitro* gene banks
2. A digitized inventory of plant resources. Part II. Other economically important spices
3. Improvement of selected spice crops through biotechnological approaches
4. On farm evaluation of tissue culture derived black pepper plants

5. Molecular characterization of viruses causing stunted disease in black pepper and development of PCR based methodology for their detection
6. Endophytic bacteria for biological system management of *Radopholous similis*, the key nematode pest of black pepper
7. Distributed Information Sub-Centre

**National Agricultural Technology Project**

1. Collection, characterization and conservation of spices genetic resources
2. Integrated National Agricultural Resources Information System
3. Agricultural Technology Information Centre
4. Prioritization Monitoring and Evaluation

**Department of Agriculture and Co-operation**

1. Centrally sponsored scheme: Integrated programme for development of spices

**Government of Kerala**

1. Technology mission on black pepper

**Board of Research on Nuclear Science**

1. Migration of pesticides in soil, water and plant environment

**International Foundation for Science**

1. Development of disinfection technology for management of bacterial wilt of ginger

**Kerala Research Programme for Local Level Development**

1. Integrated disease management in black pepper - A study on technology diffusion and impact

## *Consultancy and Commercialization of Technologies*

This year also the Consultancy Processing cell has taken up various consultancies relating to analysis of macro, micro and trace elements in soil, plant, manure and fertilizer samples; enumeration of microbes such as *Trichoderma* spp., *Pseudomonas* spp., *Azospirillum* spp. etc. and estimation of essential oils. Scientists of the institute have visited few plantations as consultants. Tech-

nology for large scale multiplication of *Trichoderma harzianum* has been sold to six new entrepreneurs. M. Sc. project work programmes of post graduate students were also taken up by the institute during the year and the institute earned Rs. 3,51,500/- through this programme. The institute also earned Rs. 5,98,000/- during 2003-04 through consultancy processing cell.

# *Institute Management Committee*

Director, Indian Institute of Spices Research, Calicut.	Chairman
Assistant Director General (PC), Indian Council of Agricultural Research, New Delhi	Member
Director of Agriculture (Kerala), Thiruvananthapuram	Member
Director of Horticulture (Karnataka), Bangalore	Member
Dr. E. V. Nybe, Associate Professor and Head, Kerala Agricultural University, Trissur.	Member
Senior Finance and Accounts Officer, Central Marine Fisheries Research Institute, Kochi.	Member
Mr. Mukundan, Kadali Kadanad P. O., Vaduvanchai, Wyanad	Member
Mr. Dhirendra Bahadur Singh, 538/28, Yogi Nagar, Ahivaranpur, Sitapur Road, Lucknow	Member
Dr. M. N. Venugopal, Head in Charge, Cardamom Research Centre, Indian Institute of Spices Research, Appangala.	Member
Dr. M. Anandaraj, Head in Charge, Division of Crop Protection, Indian Institute of Spices Research, Calicut.	Member
Dr. B. Chempakam, Head in Charge, Division of Crop Production and Post Harvest Technology, Indian Institute of Spices Research, Calicut.	Member
Dr. B. Sasikumar, Senior Scientist, Indian Institute of Spices Research, Calicut.	Member
Assistant Administrative Officer, Indian Institute of Spices Research, Calicut.	Member Secretary

# Research Advisory Committee

Dr. K. V. Ahamed Bavappa	: Chairman
Prof. V. Arunachalam	: Member
Dr. C. K. George	: Member
Dr. R. K. Sharma	: Member
Mr. C. B. S. Rajput	: Member
Dr. V. A. Parthasarathy	: Member
Dr. K. V. Ramana	: Member Secretary

The Research Advisory Committee meeting was held during 9–10 April 2003 at Calicut (Fig. 22) and the following recommendations were made.



Fig. 22. RAC meeting in progress

## Mega Project 1

1. For germplasm collection, specific areas for survey have to be identified.
2. An alternate area has to be identified for germplasm conservation as the valuable materials now conserved at IISR Farm, Peruvannamuzhi are prone to damage by wild animals.
3. Parameters (standards) with ranges for all the characters such as yield, quality,

resistance to pests, diseases, drought etc. have to be defined clearly for guidance in evaluating the germplasm of different crops.

4. Too much dependence on RAPD characterization is not advisable due to lack of consistency. Alternate and more efficient molecular markers may be located and used.
5. The earlier decision that research on paprika has to be done through AICRPS may be implemented.
6. While characterizing the germplasm, the stability of all the characters in each cycle of regeneration has to be monitored.
7. Possibility to achieve seed set in ginger may be explored.

## Mega Project II

1. Growth regulators may be used for obtaining high success in grafting and budding in tree spices.
2. Breeding programmes in ginger and turmeric other than clonal selection have to be given more importance.
3. Molecular characterization and marker-assisted breeding must be given greater importance to strengthen the established breeding methods.
4. Cultivation of cassia at higher altitudes may be encouraged.

## Mega Project III

1. Organic production of spices has to be demonstrated at the institute farm and in farmers' fields around

Peruvannamuzhi in collaboration with KVK.

2. Whenever experiments on micronutrients are conducted, basic data on the availability of these nutrients in the soil has to be estimated so that an objective evaluation of the efficacy of the added nutrients can be made.
3. Cost benefit ratio has to be worked out in the experiments on biofertilizers.

#### Mega Project IV

1. Parameters for characterization have to be fixed and this has to be given priority than adding more physiological and molecular parameters.

#### Mega Project V

1. On-farm grading has to be standardized on a priority basis.
2. Post harvest processing and value addition programmes are to be reoriented based on expert opinion from NIN and other related agencies.

#### Mega Project VI

1. Training the farmers in the production of quality planting materials through KVK may be intensified.
2. A large quantity of planting materials were produced and supplied to different agencies. It is vital to collect the information on returns obtained by them and a data base to be constructed. This project should be an avenue to get land to lab feed back.

#### Mega Project VII

1. The basic data on distribution, incidence and crop losses due to major diseases, pests and nematodes has to be up dated and a list of those requiring greater attention prepared.
2. Varietal reaction to viral diseases in black pepper has to be worked out.
3. Cytokinins may be tried for preventing spike shedding in black pepper.

4. Interim recommendations to contain the viral diseases in black pepper have to be given.

#### Mega Project VIII

1. In view of the time and cost intensity of molecular approaches, it is essential to identify parameters to decide on first and higher stages of selection for resistance over the work already done. Molecular approaches need to be carefully chosen only when other alternatives are absent/insufficient/cost intensive etc.
2. As far as possible larger number of germplasm may be screened at a time at a given location so that the data obtained are comparable and valid.
3. Most virulent strains of pathogens have to be used in screening.

#### Mega Project IX

1. The efficacy of cultural practices combined with zero tillage, particularly for the management of *Phytophthora* foot rot disease in black pepper may be studied.
2. Management of bacterial wilt of ginger through rhizome solarization should be demonstrated in farmer's fields at different locations.
3. Technologies for mass production of efficient strains of biocontrol agents have to be developed and popularized.

#### Mega Project X

1. Economics and statistics should be better integrated with relevant programmes of crop improvements so that cost-benefit and other economic evaluation and interpretation of experiments can strengthen the programme.
2. Cost of production of important crops has to be updated.
3. Full details of the technologies developed should not be put in the website and internet.

**Mega Project XI**

1. Directors of Agriculture/Horticulture of state governments may be contacted to nominate the officials for institute's diverse training programmes.
2. Success stories from the farmers may be popularized among other farmers through mass media and it should be made available in the Institute's website.
3. Success stories of the institute should also be made available at the institute website.
4. There is scope and need for testing various technologies developed by the institute in farmers' field. Operational and knowledge manuals have to be prepared and selected farmers trained in efficient systems of spices production who in turn will train other farmers.



## *Staff Research Council*

The XVII Annual Research Council meeting of the Indian Institute of Spices Research, Calicut was held on 11 April 2003 at Calicut. Dr. V. A. Parthasarathy, Director delivered the inaugural address.

The existing research projects of the institute were regrouped under 11 mega projects and project leaders were *identified for the same*. Two ongoing projects were kept in abeyance, six projects were closed and four new projects were approved during the year. At present, 38 projects are being undertaken at the institute.

Three high yielding, high quality black pepper lines were approved for state release. Two technologies for transfer to extension agencies namely, Plant growth promoting rhizobacteria (PGPR) for black pepper and Coconut coir pith as a carrier medium for biocontrol agents were discussed and approved.

The mid term review of research projects in

progress at the institute was held during 18–19 November 2003 at Calicut. The meeting was chaired by Dr. V. A. Parthasarathy, Director, and the technical sessions were co-chaired by Mr. B. Krishnamoorthy, Head in Charge, Division of Crop Improvement; Dr. B. Chempakam, Head in Charge, Division of Crop Production and Post Harvest Technology; Dr. M. Anandaraj, Head in Charge, Division of Crop Protection and Mr. K. Narayana Kurup, Head in Charge, Section of Social Sciences. The progress of work in the research projects of the institute was reviewed. One new project was presented and approved, two projects which were kept in abeyance were revived, and one project was closed during the review meeting. A meeting of the Administrative and Accounts officers with scientists was held to discuss some of the problems faced by scientist in administrative matters.

# *Participation of Scientists in Meetings*

Regional Workshop on Coffee Based Cropping System for Kerala, Regional Coffee Research Station, Coffee Board, Chundale, 6 April 2003 (K. Kandiannan).

Meeting of High Power Committee on IT and BT, Govt. of Kerala, Thiruvananthapuram, 12–13 April 2003 (V. A. Parthasarathy).

National Consultative Meeting on Improvement in Productivity and Cultivation of Ginger, Directorate of Horticulture and Ministry of Agriculture, Govt. of India, Aiswal, Mizoram, 12–14 May 2003 (V. A. Parthasarathy).

National Seminar on Floriculture, ICAR Research Complex, Barapani, Meghalaya, 14–15 May 2003 (V. A. Parthasarathy).

Action Plan Meeting of KVKs of Zone VIII, Bangalore, 14–15 May 2003 (T. K. Jacob).

National Seminar on Bioinformatics and Biodiversity Data Management, Tropical Botanical Garden and Research Institute, Thiruvananthapuram, 15–17 May 2003 (K. V. Saji).

Brain Storming Session on Proteomics and Its Applications, Indian Institute of Spices Research, Calicut, 24 May 2003 (K. N. Shiva).

32<sup>nd</sup> Town Official Language Implementation Committee Meeting, Calicut, 28 May 2003 (B. Krishnamoorthy).

Institute Management Committee Meeting, Central Plantation Crops Research Institute, Kasaragod, 7 June 2003 (V. A. Parthasarathy).

Interactive Meeting to Discuss on the Strategies for DNA Finger Printing, Molecular Characterization and Genomics, Tata Energy Research Institute, New Delhi, 17 June 2003 (K. Nirmal Babu).

Executive Meeting of DISC of CPCRI and IISR, Central Plantation Crops Research Institute, Kasaragod, 19 June 2003 (V. A. Parthasarathy).

Brainstorming Session on Database for the Management of Genetic Resources of Horticultural Crops, Central Plantation Crops Research Institute, Kasaragod, 19 June 2003 (V. A. Parthasarathy, M. Anandaraj, Johnson K. George, Santhosh J. Eapen).

Seventh Executive Development Programme in Agriculture Research Management, National Academy of Agricultural Research Management, Hyderabad, 17–23 July 2003 (V. A. Parthasarathy).

Seminar on Chemistry in the New Millennium, National Institute of Technology, Calicut, 24–25 July 2003 (N. K. Leela).

Zonal Workshop of National Agricultural Technology Project on Plant Biodiversity, Central Tuber Crops Research Institute, Trivandrum, 5–6 August 2003 (K. V. Saji).

Seminar on Turmeric, Hingoli, Maharashtra, 8 August 2003, (B. Sasikumar).

Workshop for Agriculture - Grassroot Level Support System for Agriculture Development at Panchayat Level, Calicut, 20 August 2003 (R. Suseela Bhai).

Seminar on Biotechnology for Rural Development Growth and Empowerment, South Malabar Gramin Bank, Olavanna, Calicut, 21 August 2003 (B. Sasikumar).

Farmers - Scientists Interaction Workshop on Development of Homestead Farming, Indian Institute of Spices Research, Calicut, 28 August 2003 (B. Sasikumar).

33<sup>rd</sup> Meeting of Scientific Advisory Commit-

tee on Krishi Vigyan Kendra, Gonikopal, 14 September 2003 (S. J. Ankegowda).

Workshop on Biotechnological Interventions in Agriculture, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, 17 September 2003 (B. Sasikumar).

Hindi Workshop on Noting and Drafting, Indian Institute of Spices Research, Calicut, 17 September 2003 (K. N. Shiva).

Workshop on Horticultural Crops: Floriculture and Plantation Crops, National Bank for Agricultural and Rural Development, Madikeri, 18 September 2003 (S. J. Ankegowda).

Review of Projects of ICAR in Zone VIII, Indian Institute of Spices Research, Calicut, 22–24 September 2003 (P. Rajeev).

Sixth International Plant Growth Promoting Rhizobacteria (PGPR) Workshop, Calicut, Kerala, 5–10 October 2003 (All Scientists).

National Symposium on Improving Crop Productivity in an Ecofriendly Environment: Physiological and Molecular Approaches, College of Basic Sciences and Humanities, Pantnagar, 15–17 October 2003 (S. J. Ankegowda).

Meeting on *Jaiva Keralam* - Preparation of Action Plan for Organic Farming, Directorate of Agriculture, Thiruvananthapuram, 23 October 2003 (B. Sasikumar, V. Srinivasan).

Research and Extension Workers' Interface, Department of Agriculture, Calicut, 27 October 2003 (B. Sasikumar).

Workshop on Agricultural Bioinformatics, Indian Institute of Spices Research, Calicut, 29–30 October 2003 (All Scientists).

Planters' Meeting, Calicut, 2 November 2003 (V. A. Parthasarathy).

33<sup>rd</sup> Town Official Language Implementation Committee Meeting, Calicut, 5 November 2003 (B. Krishnamoorthy).

Second State Level Research Extension Interface, Thiruvananthapuram, 5–6 November 2003 (S. Devasahayam).

National Symposium on Recent Advances in Indian Floriculture, Kerala Agricultural University, Trissur, Kerala, 12–14 November 2003 (K. N. Shiva).

National Seminar on New Perspectives of Spices, Medicinal and Aromatic Plants, ICAR Research Complex, Goa, 27–29 November 2003 (V. A. Parthasarathy, M. Anandaraj, B. Chempakam, T. John Zachariah, B. Sasikumar, Johnson K. George, C. K. Thankamani, R. Suseela Bhai, R. Ramakrishnan Nair, K. Kandianan, N. K. Leela, K. V. Saji, V. Srinivasan).

Ginger Meeting, Department of Horticulture, Gangtok, 1–3 December 2003 (A. Kumar).

10<sup>th</sup> Congress of Asian and Oceania Biochemists and Molecular Biologists, Indian Institute of Science, Bangalore, 7–11 December 2003 (B. Chempakam).

Brainstorming Session on Utilization of Genetic Resources, New Delhi, 11 December 2003 (V. A. Parthasarathy).

National Seminar on Physiological Interventions for Improved Crop Productivity and Quality: Opportunities and Constraints, S.V. Agricultural College, Tirupati, 12–14 December 2003 (S. J. Ankegowda).

Symposium on Recent Developments in the Diagnosis and Management of Plant Diseases for Meeting Global Challenges, University of Agricultural Sciences, Dharwad, 18–20 December 2003 (R. Suseela Bhai, A. Kumar).

VIII ICAR Governing Body Meeting, New Delhi, 24 December 2003 (V. A. Parthasarathy).

VIII ICAR Regional Committee Meeting, Bangalore, 29–30 December 2003 (V. A. Parthasarathy).

Brainstorming Session on Bioinformatics, Anna University, Chennai, 5 January 2004 (Santhosh J. Eapen).

National Seminar on Biotechnology - A tool for Sustainable Agricultural Production, B. A. College of Agriculture, Anand, Gujarat, 5–6 January 2004 (B. Sasikumar).

XV BTISNet Annual Coordinators Meeting, Anna University, Chennai, 6-7 January 2004 (Santhosh J. Eapen).

Sixteenth Kerala Science Congress, Centre for Water Resources Development, Calicut, 29-31 January 2004 (P. A. Mathew, B. Krishnamoorthy, K. Nirmal Babu, V. Srinivasan).

7<sup>th</sup> Conference on Global Spatial Data Infrastructure, Bangalore, 30 January-16 February 2004 (Utpala Parthasarathy).

XVII National Group Meeting of Research Workers of AICRP on Spices, Calicut, 3-5 February 2004 (All Scientists).

NATP Meeting, Central Tuber Crops Research Institute, Thiruvananthapuram, 13 February 2004 (V. A. Parthasarathy).

Farmers' Seminar, Calicut Flower Show 2004, Calicut, 13 February 2004, (B. Sasikumar).

Conference on Organic Agriculture in North East, ICAR Research Complex, Barapani,

Shillong, 16 February 2004 (V. A. Parthasarathy).

Meeting on Seed Production in Agricultural Crops and Fisheries, National Bureau of Plant Genetic Resources, New Delhi, 23-25 February 2004 (V. A. Parthasarathy).

ICAR Meeting on Molecular Mapping in Crop Plants, National Bureau of Plant Genetic Resources, New Delhi, 27 February 2004 (K. Nirmal Babu).

ICAR Meeting on Availability and Utilization of Genes/Constructs Related to Development of Transgenics in Field and Horticultural Crops, National Bureau of Plant Genetic Resources, New Delhi, 28 February 2004 (K. Nirmal Babu).

Director's meeting of Horticulture Division, Indian Council for Agricultural Research, New Delhi, 11 March 2004 (V. A. Parthasarathy).

Workshop on Networking of Indian Biodiversity Databases, Centre for Ecological Sciences, Bangalore, 23-24 March 2004 (Santhosh J. Eapen).

## Meetings Organized by the Institute

Brain storming session on Proteomics and its Applications, 24 May 2003.



Fig. 23. XVII National Group Meeting of All India Co-ordinated Research Project on Spices in progress

Workshop on Noting and Drafting in Hindi, 17 September 2003.

Annual Review Meeting of Transfer of Technology Centres of Zone VIII, 22–23 September 2003.

VI International Plant Growth Promoting Rhizobacteria (PGPR) Workshop, 5–10 October 2003.

Workshop on Agricultural Bioinformatics, 29–30 October 2003.

XVII National Group Meeting of All India Coordinated Research Project on Spices, 3–5 February 2004 (Fig. 23).

## *Radio Talks*

Integrated pest and disease management in pepper, All India Radio, Madikeri, 26 June 2003 (M. N. Venugopal).

Control of mealy bug and little leaf disease in pepper, All India Radio, Kozhikode, 14 August 2003 (V. K. Saira Banu).

Harvesting and processing of ginger and turmeric, All India Radio, Kozhikode, 17 November 2003 (K. M. Prakash).

Cardamom curing and processing, All Indian Radio, Madikeri, 23 November 2003 (S. J. Ankegowda).

Baking of foods, All India Radio, Kozhikode, 1 December 2003 (Lixy John).

Safed musli, All India Radio, Kozhikode, 13 December 2003 (B. Sasikumar).

Diversified use of spices, All India Radio, Kozhikode, 4 February 2004 (T. J. Zachariah).

Genetic resources of spices – Spices for prosperity, All India Radio, Kozhikode, Trissur and Thiruvananthapuram, 13 February 2004 (B. Sasikumar).

Agar wood - a new tree spice, All India Radio, Kozhikode, 25 February 2004 and 8 March 2004 (B. Sasikumar).

Diversified use of spices (question and answers), All India Radio, Kozhikode, 23 March 2004 (T. J. Zachariah).

Varieties of tree spices (interview), All India Radio, Kozhikode, 29 March 2004 (B. Krishnamoorthy).

## *Lectures Delivered by Scientists*

Tree spices cultivation in coffee plantation. Regional Coffee Research Station, Chundale, 6 April 2003 (K. Kandiannan).

Cost economics of major spices, Tamil Nadu Agricultural University, Coimbatore, 21 May 2003 (B. Krishnamoorthy).

Value added products from spices, Training for Farmers, Kannur, 28 May 2003 (T. John Zachariah).

Value addition in pepper and other spices, Farmer's Meeting, Vadakara, 9 July 2003 (T. John Zachariah).

Microbial genetics, Seminar on Biotechnology, Providence Women's College, 16 August 2003 (A. Kumar).

Spices cultivation in coconut and arecanut garden with special emphasis on black pepper and vanilla, State Farmer's Day, Malappuram, 17 August 2003 (S. Hamza).

An introduction to bioinformatics, Training on bioinformatics, Central Plantation Crops Research Institute, Kasaragod, 18 August 2003 (Santhosh J. Eapen).

Production management in black pepper, Training for Agricultural Officers of Calicut and Wyanad Districts, Feroke, 23 August 2003 and 23 October 2003 (P. Rajeev).

Organic farming in spices, Research-Extension Interface, Calicut, 29 August 2003 (V. Srinivasan).

Diseases of vanilla, Seminar on Vanilla Cultivation, Calicut, 1 September 2003 (R. Suseela Bhai).

Diseases of vanilla, Seminar on Vanilla Cultivation, Kannur, 13 September 2003 (R. Suseela Bhai).

'WWW, HTML and URLs' - Bioinformatics 'A' Level Course, DOEACC Centre, Calicut, 18 September 2003 (Santhosh J. Eapen).

Soil chemistry, plant and nutrition. PSMO College, Malappuram, 10 October 2003 (S. Hamza).

Pests and diseases of black pepper and vanilla, Farmer's Seminar, Pulpally, Wyanad, 31 October 2003 (Santhosh J. Eapen).

Cultivation practices in black pepper, cardamom and vanilla, Farmer's Meeting, 21 November 2003 (S. J. Ankegowda).

Microarray technology and its application in plant virus diagnosis, Winter School on Detection of Plant Virus by PCR and Nucleic Acid Probes, Tamil Nadu Agricultural University, Coimbatore, 21 November 2003 (A. Ishwara Bhat).

Cultivation practices of cardamom, black pepper, ginger and vanilla, Training for Teachers, Napokkalu, 27 November 2003 (S. J. Ankegowda).

Molecular characterization of plant pathogens and biocontrol agents, Winter School on Biotechnological Approaches for the Management of Plant Pathogens in Export Oriented Horticultural Crops, Tamil Nadu Agricultural University, Coimbatore, 19 December 2003 (M. Anandaraj).

Transgenic plants resistant to viruses: a perspective, Winter School on Biotechnological Approaches for the Management of Plant Pathogens in Export Oriented Horticultural Crops, Tamil Nadu Agricultural University, Coimbatore, 19 December 2003 (A. Ishwara Bhat).

Chemistry of spices, Inaugural Function of Chemistry Association, Providence Women's

- College, Calicut, 9 January 2004 (T. John Zachariah).
- Soil fertility and plant nutrition with emphasis on organic cultivation practices. Seminar on Organic Farming, Calicut, 13 January 2004 (S. Hamza).
- Crop production and plant nutrition. Training for Farmers, Malappuram, 15 January 2004 (S. Hamza).
- Cultivation of black pepper, Officers of Coffee Board and Horticulture Department, Kodagu, 20 January 2004 (M. N. Venugopal).
- Cultivation of vanilla, Officers of Coffee Board and Horticulture Department, Kodagu, 21 January 2004 (M. N. Venugopal).
- Pepper production management, Farmer's Training on *Jalanidhi* Project, Calicut, 23 January 2004 (P. Rajeev).
- Scientific awareness in society, Students of VHSS for Girls, Calicut, 28 February 2004 (T. John Zachariah).
- Introduction to physical biochemistry to bioinformatics, Students of Department of Electronic Accreditation of Computer Courses, Calicut, 3 March 2004 (T. John Zachariah).
- Black pepper and vanilla cultivation practices in village level horticulture, Training for Farmers, Napokkalu, 9 March 2004 (S. J. Ankegowda).
- Agronomical practices in black pepper cultivation, Seminar on Black pepper, Madikeri, 16 March 2004 (S. J. Ankegowda).
- Black pepper, Training to Coffee Growers, Kodagu, 16 March 2004 (M. N. Venugopal).
- Phytophthora* research in horticultural crops, Refresher Course in Plant Pathology, Chidambaram, 22 March 2004 (M. Anandaraj).
- Gene finding and tagging strategies, Seminar on Bioinformatics for Biosciences, GRD College of Science, Coimbatore, 22 March 2004 (Johnson K. George).
- Spice based products to entrepreneurs, Entrepreneurs Meet, Calicut, 31 March 2004 (T. John Zachariah).



## *Distinguished Visitors*

Dr. K. V. Ahamed Bavappa, Former FAO Consultant and Former Director, Central Plantation Crops Research Institute, Kasaragod.

P. Balachandran, Passport Officer, Kozhikode.

Dr. B. Bashishtha, Director, National Research Centre on Seed Spices, Ajmer.

Shri. Hukkumdeo Narayan Yadav, Hon. Minister of State for Agriculture, Govt. of India (Fig. 24).



Fig. 24. Shri. Hukkumdeo Narayan Yadav, Hon. Minister of State for Agriculture in a discussion with scientists

Mr. Joseph Thekke Kuruvinal, Chief Judicial Magistrate, Kozhikode.

Dr. G. Kalloo, Deputy Director General (Horticulture), Indian Council of Agricultural Research, New Delhi.

Dr. S. N. Pandey, Assistant Director General (PC), Indian Council of Agricultural Research, New Delhi.

Dr. K. V. Peter, Vice Chancellor, Kerala Agricultural University, Trissur.

Dr. R. B. Rai, Director, Central Agricultural Research Institute, Port Blair.

Mr. V. S. Ramachandran, Director, Regional Science Centre, Calicut.

Dr. P. S. Rao, Vice President, Indo-American Hybrid Seeds, Bangalore.

Dr. Renu Swarup, Director, Department of Biotechnology, New Delhi.

Dr. R. K. Sharma, Former Dean, Rajasthan Agricultural University, Jobner.

Dr. S. K. Sharma, Project Director, Project Directorate for Cropping System Research, Modipuram.

Dr. V. A. Srinivasan, Executive Director, Indian Immunologicals Ltd., Hyderabad.

Dr. I. V. Subba Rao, Former Vice Chancellor, ANGRAU, Hyderabad.

# Empowerment of Women

A Women's Cell is functioning at IISR to cater to the welfare and solve grievances of women employees working in the Institute. The Women's Cell conducted various programmes for the welfare, development and empowerment of farm and rural women in collaboration with the Krishi Vigyan Kendra (KVK). The International Women's Day was celebrated on 8 March 2004. The meeting was addressed by Dr. Lalitha, State President, Women's Wing, Indian Medical Association, who stressed the need for uplifting women for the betterment of society and nation.

KVK conducted various training programmes for farm women and women Self Help Groups (SHGs) in association with Departments of Agriculture, Animal Husbandry and voluntary organizations such as Centre for Overall Development, Vikas Voluntary Vahini (VVV) clubs, Indian Farmer's Movement (INFAM) etc. The training programmes mainly focused on self employment and additional income generation for women. Various need based vocational training programmes for women SHG's were organized for quality improvement in various enterprises.

During the period, 36 training programmes were organized for the farm women and women SHG's in which 654 trainees participated (Fig. 25). The programmes covered various aspects of agriculture horticulture, animal husbandry and handicrafts as indicated below:

## 1. Seed production and establishment of nurseries

- a. Organic farming (27 participants)



Fig. 25. A training programme on production of vermicompost for women entrepreneur

- b. Production of vermicompost (21 participants)
  - c. Training on plant propagation (39 participants)
  - d. Cultivation of vanilla (99 participants)
  - e. Cultivation of cowpea (12 participants)
  - f. Cultivation of bittergourd (9 participants)
  - g. Cultivation of azolla (14 participants)
- ## 2. Rearing and care of poultry and live-stock
- a. Management of milch cow (43 participants)
  - b. Goat rearing under rural conditions (12 participants)
  - c. Backyard poultry rearing (25 participants)
  - d. Management of diseases in cattle (25 participants)
  - e. Control of plant poisoning in dairy animals (61 participants)
- ## 3. Cottage industries
- a. Handicrafts making from bamboo (13 participants)

- b. Hand embroidery (14)
- c. Fabric painting and card making (18 participants)
- d. Advanced training in fabric painting (10 participants)
- e. Tablemat and artificial flower making (28 participants)
- f. Paper bag making (11 participants)
- g. Production of mushroom (36 participants)
- h. Apiculture (11 participants)
- i. Preparation of cutlet (12 participants)

**KVK** also conducted a frontline demonstration programme on production of organic cowpea in two women farmers' fields, and an on-farm testing programme on management of fruitfly in bittergourd in three women farmers' fields.

As a result of the various training programmes conducted, several self employment units were started by women in vermicomposting, bamboo handicraft making, poultry and goaterly etc. earning reasonable income through the sale of products. Sale of vermin compost and vermin wash produced by the trained SHG members was also promoted through **INFAM**.

# Personnel

Headquarters, Calicut

## *Managerial*

Dr. V. A. Parthasarathy, Director

## *Scientific*

Dr. K. V. Ramana, Project Coordinator

Dr. K. Narayana Kurup, Principal Scientist (Agricultural Statistics) and Head in Charge, Section of Social Sciences

Dr. M. Anandaraj, Principal Scientist (Plant Pathology) and Head in Charge, Division of Crop Protection

Dr. B. Chempakam, Principal Scientist (Plant Biochemistry) and Head in Charge, Division of Crop Production and Post Harvest Technology

Dr. S. Devasahayam, Principal Scientist (Agricultural Entomology)

Mr. B. Krishnamoorthy, Principal Scientist (Plant Breeding) and Head in Charge, Division of Crop Improvement and Biotechnology

Dr. K. Nirmal Babu, Senior Scientist (Plant Breeding)

Dr. M. S. Madan, Senior Scientist (Agricultural Economics)

Dr. T. John Zachariah, Senior Scientist (Plant Biochemistry)

Dr. B. Sasikumar, Senior Scientist (Plant Breeding)

Dr. J. Rema, Senior Scientist (Horticulture)

Dr. Johnson K. George, Senior Scientist (Genetics and Cytogenetics)

Dr. C. K. Thankamani, Senior Scientist (Agronomy)

Dr. R. Suseela Bhai, Senior Scientist (Plant Pathology)

Dr. A. Ishwara Bhat, Senior Scientist (Plant Pathology)

Dr. R. Ramakrishnan Nair, Senior Scientist (Genetics and Cytogenetics)

Dr. P. Rajeev, Senior Scientist (Agricultural Extension)

Dr. K. S. Krishnamurthy, Senior Scientist (Plant Physiology)

Dr. K. Kandiannan, Senior Scientist (Agronomy)

Mr. K. M. Abdulla Koya, Scientist (Selection Grade) (Agricultural Entomology)

Dr. Santhosh J. Eapen, Scientist (Selection Grade) (Nematology)

Dr. N. K. Leela, Scientist (Selection Grade) (Organic Chemistry)

Mr. K. V. Saji, Scientist (Senior Scale) (Economic Botany)

Dr. A. Kumar, Scientist (Senior Scale) (Plant Pathology)

Dr. V. Srinivasan, Scientist (Senior Scale) (Soil Science)

Dr. K. N. Shiva, Scientist (Senior Scale) (Horticulture)

## *Technical*

Dr. Johny A. Kallupurackal, Technical Information Officer (T-8)

Mr. P. Azgar Sheriff, Technical Officer (T-6)

Dr. S. Hamza, Technical Officer (T-6)

Ms. Utpala Parthasarathy, Technical Officer (T-6)

Mr. M. M. Augusthy, Technical Officer (T-5)

Mr. K. Jayarajan, Technical Officer (T-5) Statistics

*Administrative*

Mr. M. K. Sachidanandan, Assistant Finance and Accounts Officer

Mr. V. L. Jacob, Assistant Finance and Accounts Officer

Mr. S. M. Chettiar, Private Secretary

Mr. A. P. Sankaran, Assistant Administrative Officer

*Krishi Vigyan Kendra*

Ms. V. Radha, Junior Accounts Officer

Experimental Farm, Peruvannamuzhi  
*Scientific*

Mr. P. A. Mathew, Principal Scientist (Horticulture) and Head in Charge

Ms. E. Jayashree, Scientist (Agricultural Structure & Process Engineering) (joined on 1 September 2003)

*Technical*

Mr. V. K. Abubacker Koya, Farm Superintendent (T-7)

*Krishi Vigyan Kendra*

Dr. T. K. Jacob, Senior Scientist (Agricultural Entomology) and Training Organizer

Mr. P. S. Manoj, Technical Officer (T-7) (Horticulture)

Dr. S. Shanmugavel, Technical Officer T (7-8) (Veterinary Science) (Study leave from 27 October 2003)

Mr. K. M. Prakash, Technical Officer (T-6) (Agronomy)

Cardamom Research Centre, Appangala  
*Scientific*

Dr. M. N. Venugopal, Principal Scientist (Plant Pathology) and Head in Charge

Dr. S. J. Ankegowda, Senior Scientist (Plant Physiology)

Mr. D. Prasath, Scientist (Horticulture) (Study leave from 31 December 2002)

*Administrative*

Ms. Enid Savitha, Assistant Administrative Officer

# Weather

## Weather data for 2003 (Peruvannamuzhi)

Month	Rainfall (mm)	No. of rainy days	Evaporation (mm day <sup>-1</sup> )
January	0.0	0	3.94
February	30.0	6	3.37
March	17.6	2	3.34
April	283.0	15	3.15
May	165.4	7	2.70
June	836.3	21	1.99
July	808.4	28	1.80
August	672.0	26	2.11
September	173.0	10	3.87
October	315.0	17	1.91
November	44.0	4	3.18
December	10.0	1	3.29
Total	3354.7	137	34.65

## Weather data for 2003 (Appangala)

Month	Temperature (°C)	Humidity (%)	Rainfall (mm)	No. of rainy days	
	Max.	Min.			
January	28.5	14.6	67.0	000.0	00
February	31.0	16.7	71.8	000.0	00
March	32.0	17.2	69.0	027.2	02
April	32.0	19.3	73.4	087.6	10
May	30.9	20.4	74.3	000.0	00
June	27.2	18.7	85.4	411.3	14
July	24.6	18.6	95.0	621.1	31
August	24.5	18.4	95.6	498.8	19
September	25.9	18.1	92.6	156.0	13
October	28.1	17.5	90.3	196.5	11
November	28.5	15.6	76.3	000.0	00
December	29.8	10.4	79.1	000.0	00
Total	-	-	-	1998.5	100



ISBN 81-86872-20-5



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