Annual Report
2002-03

Indian Institute of Spices Research
Calicut

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Front cover
Black pepper leaf infected with badna virus (bottom-left), the vector striped mealybug (*Ferrisia virgata*) (bottom-right) and electron micrograph of the virus (top).

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Contents

Preface

I have great pleasure in presenting the Annual Report of the Indian Institute of Spices Research, Calicut, for 2002–03. We are in the beginning of the X Five Year Plan and a large number of steps have been initiated by the institute for improvement of spices, which would go a long way in benefiting spice growers and entrepreneurs in the country.

The year saw a very active HRD programme at the institute and six training programmes were conducted for scientific, administrative and supporting staff, research scholars and students. The Krishi Vigyan Kendra and the extension wing of the institute also conducted a large number of training programmes for rural youth and women, farmers and extension personnel. Two high yielding ginger varieties namely, 'Mahima' and 'Rejatha' and a clonal selection of nutmeg, 'Vishwasree' were recommended for release in Kerala. In addition, three black pepper lines were also recommended for release by the Staff Research Council of the institute. Based on the recommendations of the Research Advisory Committee, all the projects in progress at the institute were reoriented and grouped into 11 mega projects with interdisciplinary and problem oriented approaches. The meetings of the Institute Management Committee and Institute Joint Staff Council were conducted regularly and the decisions taken in these meetings were implemented.

All these developments would not have been possible but for the strong support and encouragement given by the Honourable Director General, Dr. Mangala Rai, and the Deputy Director General (Horticulture and Crop Sciences), Dr. G. Kalloo, to whom I place on record my grateful thanks. I compliment Dr. J. Rema and Dr. S. Devasahayam for the fine editing of the Annual Report. I express my gratitude to all the employees of institute for their support and cooperation.

Calicut
September 2003

V. A. Parthasarathy
Director

Executive Summary

The Indian Institute of Spices Research, Calicut, has been serving as a nodal research institute for spices for over a quarter century. The year under report marks the beginning of the X Five Year Plan period and the tentative budget allocation for the institute is 75% more under Plan and 30% more under Non Plan. The institute has initiated steps for a strong human resource development programme for staff, a multi-disciplinary approach to research programmes, effective inter-institutional collaboration and problem-oriented approaches instead of division-oriented projects. The research highlights and other major activities of the institute are summarized here.

Genetic resources

Ninety-three accessions of *Piper* species and cultivated black pepper types were collected from Kerala and Andaman Islands. One hundred and ten accessions were characterized and catalogued based on IPGRI descriptor. Twenty-six accessions of cardamom and 11 accessions of related genera were added to the germplasm. Seventy-two accessions were evaluated for yield and vegetative characters based on IPGRI descriptor. Twenty-four *Zingiber* and 20 *Curcuma* accessions were added to the germplasm. Seven collections of *Garcinia* species, a collection of *Cinnamomum sulphuratum* and six wild species of *Myristica* were added to the germplasm. Twenty accessions of black pepper, 18 accessions of turmeric, 15 accessions of ginger and 100 accessions of vanilla were added to the *in vitro* gene bank.

Characterization of germplasm

RAPD profiles of 14 major cultivars and 10 released varieties of black pepper indicated that the *Phytophthora* tolerant lines among them formed a cluster of their own. Morphological and cytological studies indicated that there was no difference between micropropagated and conventionally propagated black pepper plants. RAPD profiles of 13 species of *Eletteria* and 24 promising genotypes revealed very low polymorphism among them. Genetic fidelity analysis of micropropagated somaclones in ginger using RAPD indicated variations among them. RAPD profiles of microrhizome derived plants were uniform. Analysis of chromosome number of 16 accessions of turmeric revealed that it ranged from 2n = 61 to 2n = 80.

Crop improvement

Analysis of data on cumulative mean yield for 7 years at Valparai (Tamil Nadu) indicated the superiority of the black pepper lines Coll. 1041, HP-105 and HP-813. The black pepper lines OPKm, Coll. 1041, HP-780 and HP-1411 performed well at Peruvannamuzhi. Two high yielding, high quality ginger varieties, IISR Mahima and IISR Rejatha and a high yielding, high quality nutmeg clonal selection, IISR Viswashree, were released for cultivation in Kerala.

Two mapping populations of black pepper were developed using Panniyur-1 x Subhakara (Karimunda) and P-24 (tolerant to *Phytophthora*) x Subhakara to prepare molecular maps. DNA was isolated from 12 accessions of *Piper* spp. and 8 accessions of cultivated black pepper for determination of parental polymorphism. Differential display RT-PCR was done with RNA isolated from leaves of black pepper plants for identification of genes involved in piperine biosynthesis and other quality attributes.

Fifteen hybrid combinations were identified in cardamom as promising for yield and tolerance to leaf blight based on *per se* performance, heterosis and specific combining ability.

Evaluation for quality

The cultivated wild *Piper* germplasm were analysed for quality and Acc. 5411 recorded 31% oleoresin and 6.2% piperine, followed by Acc. 5442 with 21% oleoresin and 6% piperine. There was no variation in quality of black pepper grafted on to *P. nigrum* and *P. colubrinum* adopting various grafting methods.

Among the 150 cardamom accessions evaluated for quality, Accs. 60, 63, 75 and 273 had more than 8% volatile oil. Accs. 257, 258, 259, 277 and 325 had about 30% α-terpinyl acetate and about 25% 1,8-cineole. Among the 60 ginger accessions evaluated for quality, Gurubathani, Kozhikkalan and Accs. 121, 260, 340 and 342 had above 5.5% oleoresin; Kozhikkalan and Gurubathani had 4% crude fibre. Enhanced activity of phenylalnine ammonia lyase (PAL) was associated with curcumin levels during early stages of rhizome development in turmeric confirming the key role of PAL in initiation of curcumin biosynthesis.

Among the 30 nutmeg accessions evaluated for quality, the essential oil content in nutmeg ranged from 7.67% to 13.89% and that in mace from 7.48% to 20.99%. A9-49 recorded the highest oil content in both nut and mace. Myristicin content in nutmeg ranged from 2.26% (A11-25) to 29.60% (A11-21) and in mace from 1.70% (A11-25) to 28.68% (A9-4-11). Elemicin content in nutmeg ranged from 1.51% (A9-37) to 29.71% (A11-26) and 1.05% (A9-49) to 29.85% (A9-4-11) in mace. The colour value of paprika accessions ranged from 42 to 171 ASTA units and 33 to 176 ASTA units for indigenous and exotic germplasm collections, respectively.

Plant nutrient management

Studies on effect of Zn application on ginger indicated that application of Zn @ 5 kg/ha resulted in significant yield increase. In experiments on IPNM in ginger, higher rhizome yields were recorded in the treatments where farmyard manure, neem cake, phosphobacteria and rock phosphate were applied along with half the recommended dose of urea.

Drought management

One hundred black pepper lines were screened for drought tolerance and among them, HP-23, HP-29, HP-301 and HP-328 were identified as promising. Six cardamom genotypes were screened for drought tolerance and APG-18 was superior in withstanding stress. Screening of 28 genotypes for relative water content, specific leaf weight and stomatal count revealed significant variations. Evaluation of various soil and water conservation measures in cardamom based cropping systems indicated that planting of pineapple as live barriers in between cardamom was promising.

Disease management

Phytophthora *foot rot*

Studies on morphological and pathogenic variability of 46 *Phytophthora capsici* isolates indicated significant differences in their pathogenicity and five isolates, namely, 98-3, 98-59, 98-146, 98-173 and 02-51 were less virulent.

Seventy hybrids were screened against *P. capsici* in the greenhouse and seven accessions (HP-9, HP-117, HP-477, HP-528, HP-561, HP-599 and HP-1660) showed tolerant reaction. Among the 25 cultivars subjected for secondary screening, 3 accessions (C-888, C-1199 and C-1204) showed tolerant reaction. Sixteen Kottanadan selections were screened to confirm their tolerance and nine selections (Accs. 2420, 2425, 2426, 2428, 2432, 2433, 2466, 2535 and 2575) were tolerant.

The performance of black pepper grafts of *P. nigrum* (*cv.* Karimunda) on rootstocks of *P. colubrinum* (resistant to *P. capsici*) in farmer's field during the fifth year was satisfactory with no incidence of foot rot disease and an average yield of 1.062 kg/vine (dry) was obtained.

Evaluation of fungal and bacterial isolates (14 each) against *P. capsici* in *in vitro* studies indicated that three fungal (IISRF-559, IISRF-563 and IISRF-567) and three bacterial isolates (IISR-526, IISR-632 and IISR-655) exhibited >50% growth inhibition of *P. capsici* and also suppressed the disease under greenhouse conditions. Molasses (0.5%) was suitable for multiplication of PGPRs for field application. Decomposed coir pith compost could be effectively used as carrier media for biocontrol agents such as *Trichoderma harzianum* and *Pseudomonas fluorescens.*

Stunt disease

The association of a badnavirus with stunt disease affected leaf samples collected from Kozhikode and Wyanad districts (Kerala) was established on the basis of symptomatology, vector transmission, electron microscopy and serology. The virus was transmitted from diseased to healthy black pepper plants by grafting and by the striped mealybug (*Ferrisia virgata*). The cucumber mosaic virus (CMV) infecting black pepper was easily transmitted on to *Nicotiana benthamiana* and *N. glutinosa* by sap inoculation. A protocol was standardized for purification of CMV from tobacco.

Spike shedding

Observations on production of bisexual flowers in spikes of Panniyur-1 revealed very low percentage of bisexual flowers during August (3.9%), which could be one of the reasons for sparse setting and spike shedding in this variety particularly at high altitudes. Panniyur-1 was the most susceptible and Panniyur-5, Subhakara, Panchami, Balankotta and Kottanadan were tolerant to natural infection of anthracnose.

Rhizome rot

Two hundred and five germplasm accessions were screened against *Pythium* spp., the causal organism of rhizome rot disease. Thirty-three accessions that escaped infection in the preliminary screening were subjected to secondary screening and five accessions (Accs. 6, 17, 130, 155 and 208) were promising.

Bacterial wilt

Six new collections of *Ralstonia solanacearum* were added to the Repository of Plant Pathogens. Rep-PCR analysis grouped the isolates (28) into four major haplotypes with a similarity coefficient of 0.70 among which, two clusters caused bacterial wilt in ginger. ITS-PCR and RAPD analysis further confirmed the narrow genetic base of the bacterial wilt pathogen. A protocol was refined for isolation of DNA of *R. solanacearum* from soil. The thermal death point and time of *R. solanacearum* was determined as 45.8°C at 30 min of exposure. The time and duration of rhizome solarization was optimized. The effect of rhizome solarization on heat build up in rhizomes and consequent heat retention was also studied. Studies on storability of solarized rhizomes indicated that 1 month of storage soon after rhizome solarization did not affect germination. Over 250 ginger accessions were screened for bacterial wilt resistance and all the accessions wilted 2 months after inoculation. A few accessions that regenerated were maintained for further screening.

Minor diseases

Macrophomina sp. was identified as the causal organism of dry rot disease and *Fusarium oxysporum*, the causal organism of eye rot disease of ginger occurring during the post harvest period. *Colletotrichum* sp., *Phoma* sp. and *Pestalotiopsis* sp. were associated with leaf spot disease affected samples collected from Peruvannamuzhi, Wyanad and Kannur areas respectively, and their pathogenicity proved. Carbendazim and a combination of mancozeb-carbendazim (mancozeb 63% + Carbendazim 12%) were found to be inhibitory to *Macrophomina* sp. and *Fusarium* sp. even at 50 ppm.

Nematode management

Twenty-four accessions of ginger and 59 accessions of turmeric were screened against root-knot nematode, *Meloidogyne incognita* among which, four ginger (Accs. 79, 197, 216 and 219) and four turmeric accessions (Accs. 54, 56, 57 and 106) were promising in the preliminary screening.

Five isolates of rhizobacteria (IISR-522, IISR-528, IISR-658, IISR-853 and IISR-859) having dual nematicidal action (suppressing both *Radopholus similis* and *M. incognita*) were short-listed as promising from a collection of 291 isolates. Field evaluation of promising fungal (*T. harzianum* and *Verticillium chlamydosporium*) and bacterial (*Pasteuria penetrans*) isolates indicated that black pepper vines treated with *V. chlamydosporium* yielded the highest when compared to other treatments. The four promising fungal isolates (*V. chlamydosporium*, *T. harzianum*, *Paecilomyces lilacinus* and *Scopulariopsis* sp.) and rhizobacteria (IISR-853 and IISR-859) also reduced foliar yellowing in black pepper vines. Studies on mass multiplication of *V. chlamydosporium* on solid substrates showed that rice bran was the best.

Insect pest management

Screening of cultivars, hybrids and somaclones of black pepper accessions available in the germplasm against *pollu* beetle (*Longitarsus nigripennis*), indicated that three cultivars had less than 1% infestation on the berries.

Surveys conducted at 13 locations in Kodagu District (Karnataka), to study the distribution of root mealybug (*Planococcus* sp.) on black pepper indicated that black pepper vines in 7 locations were infested by the pest. Evaluation of microbial pathogens and entomomopathogenic nematodes (EPNs) against root mealybug indicated that EPNs caused up to 32% mortality of the pest in laboratory bioassays.

Fortnightly sprays of neem oil 1% or Nimbicidine 1% during July to October was promising for reducing the infestation of shoot borer (*Conogethes punctiferalis*) on ginger. Screening of 485 germplasm accessions of ginger against the pest indicated that 8 accessions had less than 5% shoots infested by the pest.

Evaluation of dried leaves of various plants as storage material for prevention of infestation by rhizome scale (*Aspidiella hartii*) indicated that storage of ginger rhizomes in dried leaves of *Strychnos nux-vomica* after dipping in quinalphos 0.075% was effective.

Evaluation of dried leaves of various plants for prevention of infestation by cigarette beetle (*Lasioderme serricorne*) indicated that storage of dried rhizomes in leaf powder of *Clerodendron infortunatum* in polypropylene containers was promising. Dried rhizomes of 91 accessions of ginger were screened for damage by cigarette beetle and 22 accessions were free of pest infestation.

Post harvest technology

Ten isolates of *Bacillus* sp. and *Pseudomonas* sp. were isolated from black pepper berries that were effective in conversion of black pepper to white pepper. Evaluation of various containers for storage of fresh ginger indicated that zero energy chamber (a double walled brick structure filled with sand between the walls, frequently moistened) was ideal for storing fresh ginger.

Transfer of technology

The institute organised four training programmes for field extension functionaries and research workers on production technology of spices. Three short-term training programmes on computers/ bioinformatics and a training programme on 'Molecular Biology Techniques for Horticultural Crops' were also conducted. Sixty-seven short-term research projects carried out by post graduate students from various universities were also supervised by scientists. The institute participated in 16 extension programmes organized by various institutions. *Kisan divas* was celebrated on 23 December 2002 and progressive farmers were honoured.

Fifty five thousand rooted cuttings of black pepper, 11,100 grafts of nutmeg, 28,000 seedlings and 50 kg seed capsules of cardamom were produced and distributed to farmers and other agencies. About 850 kg of *Trichoderma* was also sold to farmers and other agencies. An on-line version of extension literature was regularly updated at the institute web site on the internet.

Krishi Vigyan Kendra

The Krishi Vigyan Kendra (KVK) conducted 78 training programmes for farmers, unemployed youth and women, school drop outs and extension functionaries. The KVK conducted frontline demonstrations in farmers' fields on cultivation of high yielding coleus and composting of coir pith. The KVK organised six *kisan melas* for farmers. The Animal Clinic took up consultancy, advisory and home services. Several rural youths generated self-employment in agri-nurseries, vermi-composting, fruit processing and goatary with the help of the KVK.

Information services

A web page, <www.spicestat.org> giving details of area, production, export and other statistics of major spices was launched and two softwares were developed. A web enabled intranet information hub, SPICENET, with interactive user-friendly menus and links was developed and launched for the benefit of the institute staff. Networking of libraries of Central Plantation Crops Research Institute, Kasaragod, and Indian Institute of Spices Research, Calicut, was also initiated for sharing of resources and facilities.

All India Co-ordinated Research Project on Spices

The AICRPS centres strengthened their genetic resources and new germplasm collections were made in ginger, turmeric, coriander and fenugreek by the Pundibari and Dapoli centres. The cardamom clones CL-629, CL-681 and CL-730 and OP progenies D-237, CL-730, 8-4-D-11 and 7-24-D11 were identified as promising at Mudigere. The promising clones P-6, D-237 and CL-746 were drought tolerant at Mudigere. Germplasm accessions tolerant to various diseases were identified in ginger, turmeric, coriander and cumin. Evaluation of quality of turmeric accessions indicated that the SG-685 gave high dry recovery. In coriander, Jco-331 had high oil (0.45%).

Micronutrients such as boron and molybdenum influenced capsule yield in cardamom at Mudigere. A package of practices for ginger and turmeric production was standardized at Chintapalli. A fertilizer package, including application of biofertilizers, was standardized for clove and nutmeg at Yercaud. The yield and quality of coriander and fennel were increased by application of Zn, Fe, Mn and Cu. Optimum sowing periods were determined in cumin and fenugreek.

Irrigation @ 2 l water/day resulted in increased spike number, green berry yield and spike length in black pepper at Panniyur. Application of micronutrient Fe as foliar spray @ 1% significantly increased the yield of ginger at Dholi. In vegetative propagation of nutmeg, the percentage of success was high in approach grafting (90%) than in epicotyl grafting (70%).

Metalaxyl Gold MZ and *Trichoderma* sp. were effective for the management of *Phytophthora* foot rot of black pepper. Two sprays of either monocrotophos (0.05%) or dimethoate (0.05%) at fortnightly intervals after harvest of berries was effective for the management of mussel scale (*Lepidosaphes piperis*) on black pepper at high ranges of Idukki District.

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 (Fig. 1). 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 agroecosystems.

Fig. 1. Laboratory and administrative buildings of the institute

- 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. 2).

Research on various aspects of mandate crops 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 Sec-

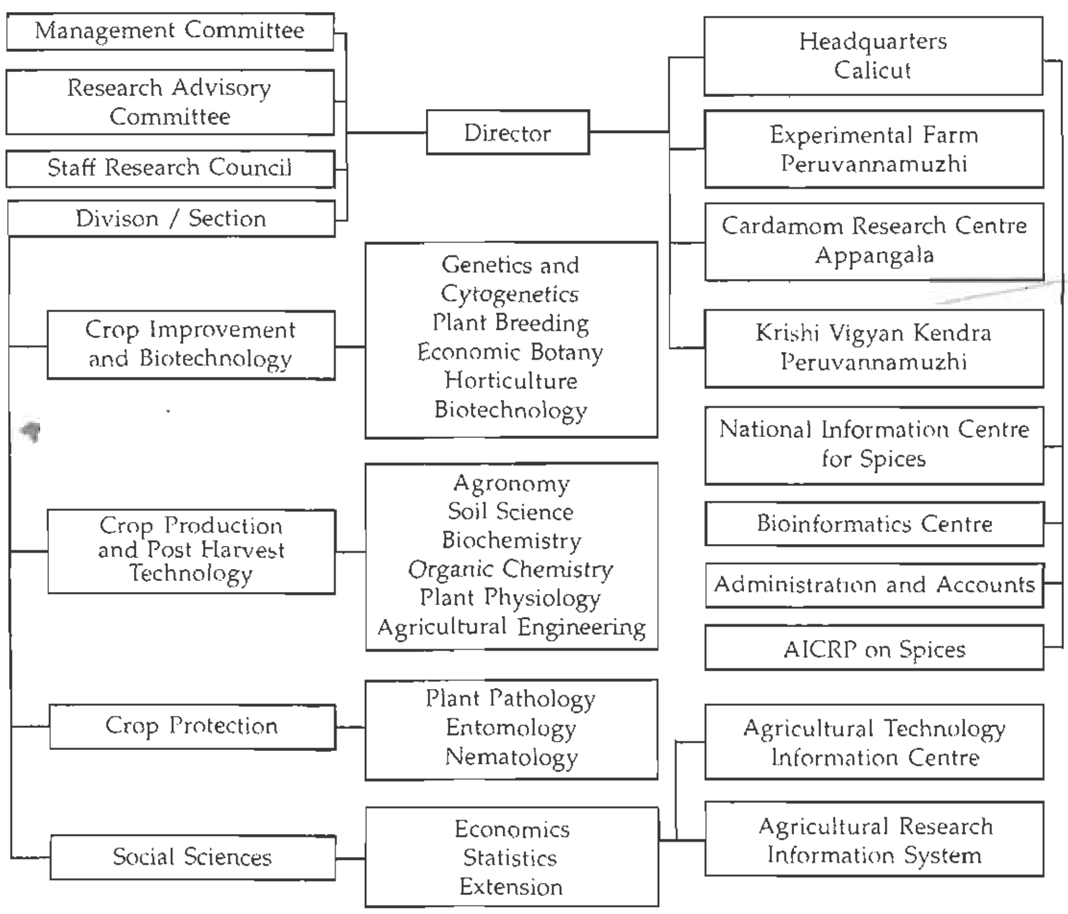

Fig. 2. Organization of IISR

tion. 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. 460 lakhs during the year which included Rs. 190 lakhs under Plan and Rs. 270 lakhs under Non Plan and the expenditure under Plan and Non Plan was 190 lakhs and 260 lakhs, respectively (Tables 1 and 2). In addition, Rs. 261.17 lakhs was also received as funds from external agencies.

Staff

The institute has a sanctioned strength of 42 scientific, 21 administrative, 37 technical and 69 supporting staff (Table 3).

Past achievements

Surveys were conducted for collection of germplasm that were conserved in germplasm conservatories and *in vitro* gene banks. The collections include 3211 black pepper, 386 cardamom, 670 ginger, 800 turmeric, 482 nutmeg, 227 clove, 302 cinnamon, 30 cassia, 32 garcinia, 180 allspice, 35 paprika and 35 vanilla accessions. The germplasm was characterized for yield, quality and resistance to pests, diseases and drought. Molecular characterization of germplasm was also initiated.

Table 1. Budget of the institute

Particulars	Amount		
	Plan (Lakh Rupees)	Non Plan (Lakh Rupees)	Total (Lakh Rupees)
Establishment	-	222.0	222.0
Travelling allowance	5.0	4.5	9.5
Works	23.0	-	23.0
Other charges	162.0	43.5	205.5
Total receipts	190.0	270.0	460.0
Expenditure	190.0	260.0	450.0

Table 2. Funds received from external agencies

Particulars	Amount (Lakh Rupees)
AICRP Spices	157.00
KVK	19.85
ICAR Cess Fund Schemes	8.44
NATP	23.30
DBT schemes	33.03
IPDS	5.55
Pension and gratuity	14.00
Total	261.17

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. Suvasini, a high yielding

Table 3. Staff position of the institute

Category	Sanctioned	In position			Vacant
		Chelavoor	Peruvannamuzhi	Appangala	
Scientific	42	27	1	3	11
Technical	37	19	13	5	-
Administration	21	15	-	2	4
Supporting	69	27	17	19	6
Total	169	88	31	29	21

variety suitable for high density planting, Avinash, a variety resistant to rhizome rot disease and Vijetha, a variety resistant to *katte* disease were released in cardamom. Three ginger varieties, Varada, Rejatha and Mahima with high yield and quality, and five high curcumin and high yielding turmeric varieties, Suvarna, Sudarsana, Suguna, Prabha and Pratibha 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 undertaken.

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 undertaken. The institute also served as a nodal agency for dissemination of information on all aspects of spice research and development.

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, B. Sasikumar, Johnson K. George, K. Nirmal Babu, D. Prasath and R. Ramakrishnan Nair)

Collection and conservation

Explorations were conducted in Kasaragod District, Peppara Wildlife Sanctuary, Neyyar Wildlife Sanctuary and Achankovil forests in Kerala; Cooch Behar and Jalpaiguri in West Bengal; Mizoram and Andaman and Nicobar Islands and 93 accessions comprising of 71 wild accessions and 22 cultivated accessions were collected. In Peppara and Neyyar, the population of *Piper attenuatum* was high when compared to other areas, probably due to the low elevation of these areas. *P. thomsoni* (a shrub with globose spikes) and *P. peepuloides* (a climber resembling *P. mullesua*, but with fused cylindrical spike at every node) were collected from Totopara forests (West Bengal) near Indo-Bhutan border. The other important collections made were *P. longum* with light purple coloured spikes from Thenmalai and *P. hapnium* (male) from Manalar area of Achankovil forests (Fig. 3).

The germplasm comprising of 862 wild accessions and 1200 cultivated accessions were maintained; 235 wild accessions and 1100 cultivated accessions were established in the field genebank and 1200 hybrids were maintained in the germplasm nursery.

Cataloguing

One hundred and ten accessions were characterized for 22 vegetative and 9 reproductive characters using MS-Access Software.

Multiplication

The germplasm accessions were multiplied and supplied to various divisions for screening against pests, diseases and drought and

Fig. 3. *Piper hapnium* collected from Achankovil forests in Kerala

also to various centers of All India Coordinated Research Project on Spices and Pepper Technology Mission for field evaluation.

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

(D. Prasath, M. N. Venugopal and K. V. Saji)

Collection and conservation

Twenty-six new accessions were collected from Idukki (15), Silent Valley (9) and Kodagu (2). The collections include a low elevation high yielding type (wander cardamom), high biomass types and local types. The field genebank at present possesses 386 accessions, which includes 11 accessions of related taxa of Zingiberaceae.

Cataloguing

Seventy-two accessions were evaluated for yield and its attributing characters (17 quantitative characters and 3 qualitative characters) based on IPGRI descriptor and two high yielding accessions namely, APG-277 and APG-279 were identified (Table 4).

Table 4. Characterization of cardamom accessions

Character	Mean	Range
Plant height (cm)	201.2	125.0–351.7
Total tillers/plant	26.6	13.5–69.7
Bearing tillers/plant	15.3	8.0–47.7
Leaves/plant	206.7	70.0–646.3
Panicles/plant	19.8	1.0–68.0
Panicle length (cm)	35.9	19.0–94.0
Nodes/panicle	19.8	14.0–37.5
Capsules/panicle	88.4	33.0–208.0
Yield/plant (g)	188.3	0.0–803.0
Seeds/capsule	15.8	9.9–21.8

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

(B. Sasikumar, Johnson K. George, K. V. Saji, K. M. Abdulla Koya and R. Ramakrishnan Nair)

Collection and conservation

Surveys were couducted at Allappady Meth (Achankovil), Neyyar, Thiruvananthapuram (Kerala); Cooch Behar, Phung Suiling, Jalpaiguri (West Bengal) and Tikamgarh (Madhya Pradesh) and 24 *Zingiber officinale* accessions including a putative wild type and a primitive cultivar from *Kani* tribes of Neyyar were collected. A *Zingiber* sp. with nutmeg rind flavour was collected from Allappady Meth. An exotic *Zingiber* species, *Z. officinale* var. *rubens* (*Kintoki*) was obtained from Japan. This accession is characterized by small lemon yellow rhizomes, comparatively dwarf stature, very less fibre (0.6%), early maturity and slight bitter taste of rhizomes.

Twenty *Curcuma longa* accessions were collected including 19 cultivated accessions from Cooch Behar, Phunt Shling and Jalpaiguri and 1 accession from Allappady Meth. At present, 691 accessions of *Zingiber* spp. and 802 accessions of *Curcuma* spp. are maintained in the field genebank.

Multiplication of released varieties

About 1000 kg seed rhizomes of Varada, 1200 kg of Mahima and 600 kg of Rejatha (ginger varieties) and 1300 kg seed rhizomes of Prabha, 1650 kg of Prathibha, 250 kg of Suguna, 275 kg of Sudarsana and 60 kg of Suvarna (turmeric varieties) were produced under nucleus seed production and distribution programme. Seed rhizomes of ginger varieties were also produced at farmer's plots at Thrissur and Wyanad.

Multiplication of ginger accessions

The putative wild type of *Z. officinale* namely, Sabarimala (Acc. 296), Kakkakalan (Acc. 558) and Kozhikalan (Acc. 557), popular cultivars of ginger such as Rio-de-Janeiro, Himachal, Maran and Suprabha and 17 selected accessions of ginger with bold rhizomes collected from Nepal, were multiplied for further evaluation for yield and quality.

Evaluation of high curcumin lines of turmeric

Eleven high curcumin lines of turmeric including seven Alleppey Finger Turmeric selections and the varieties Prabha and Prathibha (controls) were evaluated at Peruvannamuzhi for the fifth consecutive year. Accs. 126, 584 and 657 were at par with controls for fresh yield during the year and Accs. 126 and 691 recorded good dry recovery (Table 5).

Table 5. Evaluation of high curcumin turmeric lines at Peruvanamuzhi

Line	Mean fresh yield (kg/3m^2 bed)	Dry recovery (%)
Acc. 126	16.25	17.6
Acc. 295	15.30	16.3
Acc. 584	16.75	16.1
Acc. 585	15.25	17.6
Acc. 591	13.75	16.3
Acc. 593	14.75	17.5
Acc. 656	13.25	16.3
Acc. 657	16.25	17.5
Acc. 691	16.00	18.7
Prabha (Control)	14.50	18.4
Prathibha (Control)	14.00	21.0
CD ($P<0.05$)	NS	-

Effect of locations on curcumin content of turmeric

The turmeric varieties/lines namely, Prabha, Prathibha, Alleppey, Sudarsana, Suguna and Suvarna and Accs. 126, 584 and 585 were evaluated along with controls BSR-1 and BSR-2 at different locations in Karnataka (Nyamati, Harveri and Bhramasagar) and Kerala (Peruvannamuzhi) to study the effect of locations on curcumin content of turmeric. Prabha, Prathibha and Acc. 126 showed consistency for curcumin content across two or three different locations (Table 6).

Table 6. Effect of locations on curcumin content of turmeric varieties/lines

Variety/Line	Curcumin (%)			
	Kerala	Karnataka		
	Peruvannamuzhii	Nyamati	Harveri	Bhramasagar
Acc. 126	5.5	6.4	-	-
Acc. 584	5.5	4.9	-	-
Acc. 585	6.0	4.9	-	-
Suguna	5.1	3.6	-	-
Sudarsana	5.0	6.0	-	-
Suvarna	3.8	-	-	5.7
Alleppey	6.0	5.6	-	-
Prabha	6.5	5.2	5.3	-
Prathibha	6.2	6.4	4.9	-
BSR-1	-	4.8	-	-
BSR-2	-	4.2	-	-

Evaluation of quality of ginger accessions

Sixty accessions of ginger were evaluated for quality parameters. The oleoresin content ranged from 3.0% (Accs. 154 and 264) to 7.5% (Acc. 537-Kozhikalan) and the essential oil content ranged from 0.8% to 2.0%.

Shelf life of dry ginger and turmeric

Dry ginger of 91 accessions were screened against the storage pest *Lasioderma serricorne* and Accs. 162 and 220 were promising with low levels of infestation. Among the three released ginger varieties, IISR Mahima was more tolerant to storage pest attack. Among the two turmeric varieties Prabha and Prathibha, Prathibha was more tolerant to the storage pest attack.

New varieties of ginger

The 21st State Seed Subcommittee, Kerala, approved for release and notification the new ginger varieties IISR Mahima and IISR Rejatha, based on its superiority for yield and quality. IISR Mahima is resistant to root knot nematode also.

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

(*B. Krishnamoorthy, J. Rema, P. A. Mathew, D. Prasath, K. P. M. Dhamayanthi, T. John Zachariah, S. Devasahayam, S. S. Veena and M. N. Venugopal*)

Collection

Seven collections of kokum (*Garcinia indica*) including *var.* Konkan Amrutha were collected from Kasaragod District (6) and Vengurla (1) (Table 7). One collection of *Cinnamomum sulphuratum* from Peppara Wildlife Sanctuary and six wild collections of *Myristica* species from Neyyar and Achankovil were also collected.

Conservation

Three hundred and two accessions of *Cinnamomum* spp., 482 of *Myristica* spp., 223 of *Syzygium* spp., and 49 of *Garcinia* spp. were maintained in the field conservatory and nursery at Peruvannamuzhi (Fig. 4). At Chelavoor, cinnamon clones (including tissue cultured and seedlings of 10 elite lines

Fig. 4. A collection of *Garcinia indica* from the germplasm

Table 7. Fruit characters of kokum collections from Kasaragod District

Acc. no.	Av. fruit wt. (g)	Av. fruit length (cm)	Av. fruit breadth (cm)	Av. rind thickness (cm)	Av. rind wt. (g)	Av. seed wt. / fruit (g)	Seed no./ fruit (Range)
ICGG-1	31.10	3.44	4.31	0.34	16.9	2.4	1–4
ICGG-3	35.10	3.28	4.57	0.35	18.0	4.1	2–6
ICGG-4	16.17	3.00	3.07	0.28	8.9	3.2	2–4
ICGG-5	31.38	3.45	4.19	0.35	16.6	4.0	2–6
ICGG-6	20.57	3.04	3.71	0.22	11.6	3.7	2–7

and 5 species), 12 cassia accessions, 19 garcinia accessions, seedlings and clonal progenies of A11/70 (a high yielding nutmeg selection), 70 nutmeg grafts, 20 clove trees and 20 allspice trees were maintained. In trials on *in situ* grafting of promising six *G. gummi-gutta* accessions (ICGG-2, 4, 5, 6, 7 and 9) on *G. gummi-gutta* carried out during 2001–02, 44 plants have established.

Cataloguing and evaluation

Cassia

Coppicing and bark extraction were carried out in 35 progenies of 4 elite cassia lines (A-2, C-1, D-1 and D-3), in the progeny evaluation trial of elite cassia lines laid out at Peruvannamuzhi in 1997. Morphological characters such as, height, number of main shoots, number of branches, canopy width and shoot thickness were recorded for all the plants, while yield characters such as, number of harvested shoots and fresh and dry weight of bark were recorded for the 35 coppiced plants. In the progeny evaluation trial of 15 cassia accessions laid out at Appangala in 2001, plant height was recorded, which ranged from 19.4 to 68.7 cm.

Clove

Forty-nine accessions of clove from the germplasm flowered during the year at Peruvannamuzhi. The yield data for 22 accessions (negligible yield was obtained in the remaining accessions) were recorded and Acc. 58 recorded the highest yield (980 g fresh weight).

Nutmeg

The yield of 92 accessions from the germplasm was recorded (Table 8).

In the progeny trial of nutmeg elite lines, characters like height, canopy, girth, number of primary branches, number of flowers per plant and yield per plant were recorded. Progenies of elite nutmeg lines A9/20 and A9/25 performed better.

In crossing studies of nutmeg using Accs. O-3 and A9-13 (males) and A9-4-12 and A9-4 (females), only two fruits could be obtained in A9-4-12.

Release and production of high yielding varieties

A high yielding, high quality nutmeg clonal selection (A9-4) IISR Viswashree was approved for release by the 21st State Seed Sub-committee, Kerala. Around 12,000 grafts of the released variety and other elite lines were distributed to farmers. Elite lines of cassia and cinnamon were also multiplied.

Evaluation of quality in nutmeg

Among the 30 nutmeg accessions evaluated for quality, the oil content ranged from 7.67% to 13.89% and that in mace from 7.48% to 20.99%. A9-49 had the highest oil content in both nutmeg and mace. Myristicin content in nutmeg ranged from 2.26% (A11-25) to 29.60% (A11-21) and that in mace from 1.70% (A11-25) to 28.60% (A9-4-11). Elimicin content in nutmeg ranged from 1.51% (A9-37) to 29.71% (A11-26) and in mace, 1.05% (A9-47) to 29.85% (A9-4-11).

Table 8. Yield of nutmeg accessions from the germplasm

Plot	No. of accessions	Yield/Acc. (No. of fruits-range)	High yielding accessions and yield (No. of fruits)
A-4	24	6–286	113 (240), 41 (285), 12 (286)
A-9	42	20–583	20 (528), 18 (573), 1 (460), 63 (467), 13 (477), 95 (493)
A-11	26	10–515	5 (305), 10 (410), 29 (515)

Floral biology of allspice

The floral biology of allspice was studied. Flower: cyme with 73–105 flowers per cyme; days to first to last flowering in inflorescence: 17–38 days. Sepals: 4, tiny, green; petals: 4, white; anthers: numerous (68–81); stigma: single, style with one ovary. Number of fruits: 7–25 per inflorescence; dry recovery: 14.3%.

5. Collection, conservation and improvement of vanilla

(K. Nirmal Babu, K. V. Saji, S. S. Veena and R. Ramakrishnan Nair)

Production of somaclones

More than 500 somaclones of *Vanilla planifolia* were produced and are being hardened for field planting.

Production of interspecific hybrids

Interspecific hybrids between *V. aphylla* (?) and *V. planifolia* (?) were produced. Among the 50 progenies generated, none expressed the leaf character of the male parent. All the progrenies of aphylla types had only scale leaves and none exhibited the normal leaf character of *V. planifolia*.

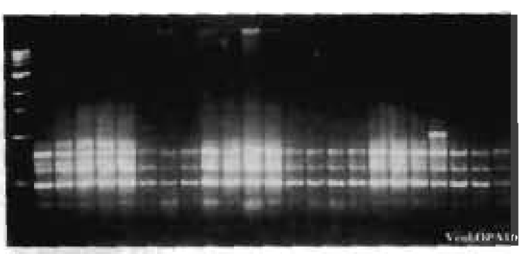

Fig. 5. RAPD profiles of vanilla collections (1. Kb ladder, 2. Yesloor, 3. Betelmana, 4. Coimbatore, 5. Saklespur, 6. ICRI, Myladumpara, 7. Calicut University, 8. Nilambur, 9. *V. marginata*, 10. Coll. 4715, 11. Coll. 4716, 12. Coll. 4717, 13. Coll. 4718, 14. Coll. 4719, 15. Coll. 4720, 16. Coll. 4723, 17. Coll. 4726, 18. Coll. 4727, 19. Coll. 4728, 20. Coll. 4729, 21. Madagascar, 22. Mauritius, 23. V. pl IJC, 24. V. pl IISR)

Molecular characterization

Molecular profiles of vanilla germplasm, including exotic and indigenous collections, seedlings, somaclones and interspecific hybrids were developed and their interrelationships were studied (Figs. 5 and 6). Studies on 23 different collections of vanilla from farmers gardens indicated certain degree of variability in their RAPD profiles. Most of the genotypes showed slight differences (0.17 coefficient of variation) and they formed two broad but distinct clusters. Among them, the collection from Madagascar was distinctly different followed by collections from Yesloor (Karnataka).

Screening against **Phytophthora** *and* **Fusarium**

Thirty-two accessions including various collections and progenies of *V. planifolia*-and related species were screened against *Phytophthora* sp. and *Fusarium* sp. Thirteen accessions showed tolerant reaction to *Phytophthora* sp., while 19 accessions showed tolerance to *Fusarium* sp. All the eight collections of *V. andamanica* and three seedling progenies of *V. planifolia* namely, V-8, V-24 and V-55 showed tolerant reaction to *Phytophthora* sp., while vanilla collection from Mauritius, progenies V-48 and V-120, *V. aphylla* and all the collections of *V. andamanica* exhibited tolerant reaction to *Fusarium* sp.

6. Development of paprika for warm humid tropics

(K. P. M. Dhamayanthi, P. A. Mathew, N. K. Leela, A. Ishwara Bhat and K. N. Shiva)

Collection and conservation of germplasm

Five accessions of paprika namely, Selection-

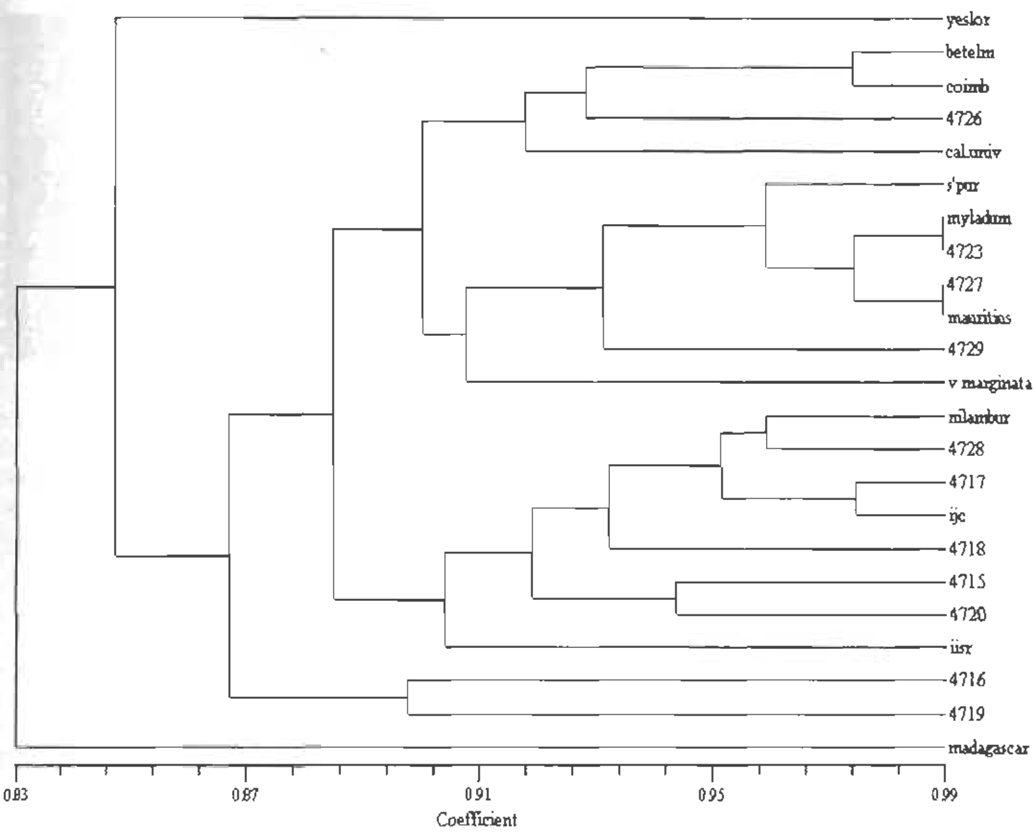

Fig. 6. Cluster analysis of vanilla germplasm

2, SSP, IMI-5, Caynne and Kt-pl-19 were collected from Indian Agricultural Research Institute, Regional Station, Katrain (Himachal Pradesh) and added to the germplasm; 40 accessions are now available in the germplasm (Fig. 7). Forty accessions including 5 new collections of paprika were raised for purification.

Analysis of quality

Colour value of 31 paprika accessions was analysed, which ranged from 42 ASTA units (Kt Pl-19) to 171 ASTA units (ICBD-13) in indigenous collections (Bydagi Dabbi-ICBD lines), and from 33 ASTA units (EC-18) to 176 ASTA units (EC-490) in exotic collections.

Fig. 7. A .collection of paprika from the germplasm

Externally Funded Projects

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

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

Exploration and collection

Explorations were undertaken in Neyyar and Peppara Wildlife Sanctuaries and Achankovil in Kerala; Andaman and Nicobar Islands; Kolasib, Aizawl, Serchip and Chempai districts in Mizoram and 158 collections were made. Seventy-nine accessions collected from Neyyar and Peppara Wildlife Sanctuaries and Achankovil include collections of *Piper, Cinnamomum, Zingiber, Curcuma, Myristica, Vanilla* and *Garcinia* species (Figs. 8 and 9). Special collections from these areas include *P. attenuatum* with hermaphrodite spikes, *V. vatsalana* from Neyyar forests, *P. longum* with light purple coloured spikes from Kulathupuzha and a collection of wild ginger from Allappady Meth and 49 ginger collections with high variability for inner core colour and fibre content from Mizoram. The important collections from Andaman and Nicobar Islands include *G. hombroniana, Curcuma* sp., a first collection of wild *Curcuma, Hornstedtia fenzlii*-a bee repellent plant belonging to the Zingiberaceae family and *V. andamanica* with self setting nature and potential for use in breeding programmes.

Fig. 8. *Piper peepuloides* collected from West Bengal

Fig. 9. *Garcinia hombroniana* collected from Andaman and Nicobar islands

Characterization

Black pepper

One hundred black pepper germplasm accessions maintained under field conditions were characterized for various qualitative and quantitative characters (Table 9).

Cardamom

Seventy accessions of cardamom were characterized and high variability for all major characters including quality was observed (Tables 10 and 11).

Ginger

Vegetative and yield characters of 100 accessions of ginger germplasm were recorded and high variability for the characters was noticed (Table 12).

Turmeric

One hundred and fifty germplasm accessions of turmeric were assessed for variability for vegetative and yield characters (Table 13).

Table 9. Characterization of black pepper accessions

Character	Mean	Range (n = 100)	
		Minimum	Maximum
Vine column height (m)	3.0	1.4	9.0
Vine column diameter (m)	3.0	0.5	5.3
Lateral branch length (cm)	34.3	12.5	60.8
No. of nodes/lateral branch	12.6	4.7	37.0
Leaf petiole length (cm)	1.6	0.9	1.0
Leaf length (cm)	13.6	8.7	22.8
Leaf width (cm)	8.3	4.9	14.2
Spike length (cm)	7.6	3.0	15.5
Peduncle length (cm)	1.2	0.3	2.7
No. of spikes/lateral branch	2.9	1.0	8.0
No. of spikes/vine	90.6	1.0	282.0
Fruit setting (%)	65.2	10.0	98.0
No. of berries in 10 spikes	321.7	95.0	690.0
No. of fully developed berries	45.1	17.0	110.0
No. of under developed berries	16.0	3.0	52.0
Fresh wt. of 100 berries (g)	12.8	6.5	21.0
Volume of 100 fresh berries (ml)	11.9	6.0	19.0
Dry wt. of 100 berries (g)	4.9	2.0	6.5
Fresh yield/standard (kg)	0.6	0.01	2.9
Fresh wt. of berries in 10 spikes	39.3	12.0	75.0
Oleoresin (%)	9.3	5.7	13.7
Essential oil (%)	3.5	2.0	6.2
Piperine (%)	2.7	1.2	5.8

Table 10. Characterization of cardamom accessions

Character	Mean	Range (n = 70)	
		Minimum	Maximum
Plant height (cm)	201.2	125.0	351.6
Total tillers/plant	26.6	13.5	69.7
Bearing tillers/plant	15.3	8.0	47.7
No. of leaves/plant	206.7	70.0	646.3
No. of panicles/plant	19.8	1.0	68.0
Panicle length (cm)	35.9	19.0	94.0
No. of nodes/panicle	19.8	14.0	37.5
No. of capsules/panicle	88.4	33.0	208.0
Fresh yield/plant (g)	188.3	0.0	803.0
No. of seeds/capsule	15.8	9.9	21.8

Table 11. *Analysis of quality of cardamom accessions*

	Malabar		Mysore		Vazhukka	
	Mean	Range	Mean	Range	Mean	Range
Essential oil (%) (v/w)	6.16	3.6–8.3	6.43	3.6–8.8	5.91	3.7–7.5
1,8-cinelole (%)	25.96	16.0–31.2	21.53	15.0–35.1	23.89	19.1–31.9
Terpinyl acetate (%)	27.41	23.6–34.4	26.81	23.1–36.8	27.20	23.2–31.4

Table 12. Characterization of ginger accessions

Character	Mean	Range (n = 100)	
		Minimum	Maximum
Plant height (cm)	96.1	72.0	130.0
Pseudostem girth (cm)	3.1	2.5	4.3
No. of tillers	15.9	5.0	39.0
No. of leaves	22.6	11.0	35.0
Average leaf length (cm)	28.0	21.1	32.3
Average leaf breadth (cm)	3.0	2.8	4.0
No. of mother rhizomes/clump	1.0	1.0	1.0
Yield/clump (g)	461.9	433.0	700.0
Essential oil (%)	1.7	1.0	2.8
Oleoresin (%)	4.3	2.5	6.6
Crude fibre (%)	2.5	1.4	4.4

Table 13. Characterization of turmeric accessions

Character	Mean	Range (n = 100)	
		Minimum	Maximum
Seedling height (cm)	53.5	31.0	75.5
Sedling girth (cm)	11.2	7.0	17.5
No. of leaves in main shoot	8.6	5.0	12.0
No. of tillers	2.5	1.0	9.0
No. of leaves in tillers	11.7	3.0	57.0
Average leaf length (cm)	63.8	38.8	90.2
Average leaf breadth (cm)	16.4	10.5	20.8
No. of mother rhizomes	2.5	1.0	7.0
Wt. of mother rhizome (g)	110.8	20.0	430.0
No. of primary rhizomes	7.4	2.0	16.0
Wt. of primary rhizome (g)	197.6	40.0	465.0
No. of secondary rhizomes	24.1	4.0	63.0
Wt. of secondary rhizome (g)	222.5	15.0	860.0
Internode length (cm)	2.6	1.1	3.5
Yield/clump (g)	520.0	70.0	1340.0
Curcumin (%)	5.6	1.4	8.5
Essential oil (%)	2.8	1.4	3.5
Oleoresin (%)	9.7	7.0	12.9

Cinnamon

One hundered accessions of cinnamon were characterized and significant variations were observed for morphological and quality characters (Table 14).

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

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

Meristems of black pepper, cardamom, ginger and turmeric were were isolated from

Table 14. Characterization of cinnamon accessions

Character	Mean	Range (n = 100)	
		Minimum	Maximum
Leaf			
Average length (cm)	12.9	8.8	20.6
Average breadth (cm)	5.3	3.3	8.3
Average ratio of LL to LB	2.7	1.9	7.9
Average size index	0.7	0.3	1.8
Yield of bark			
Fresh weight (g)	228.5	30.0	1520.0
Dry weight (g)	67.5	8.0	305.0
Bark recovery (%)	31.5	10.7	80.0
Quality			
Oleoresin (%) (bark)	8.1	0.6	19.9
Essential oil (%) (bark)	2.3	0.3	20.0
Essential oil (%) (leaf)	1.8	0.0	4.8
Cinnamaldehyde (%)	77.7	61.5	91.0
Eugenol (%)	67.3	40.7	86.0

in vitro cultures and cultured on standardized specific medium for regeneration; 50% success was obtained in cardamom, ginger and turmeric, but in black pepper, phenolic exudates resulted in death of isolated meristems.

Cryopreservation of ginger embryoids and vanilla protocorms was carried out using three different techniques namely, encapsulation dehydration, encapsulation vitrification and vitrification. Among these, vitrification was the best for ginger embryoids and encapsulation dehydration for vanilla protocorms.

Cryo preserved pollen of *Vanilla planifolia* and *V. aphylla* could result in successful fruit set and seed germination. For cryo preservation of vanilla pollen, fresh pollen was collected, desiccated for 10 min and treated with 10% DMSO for 10 min and cryo preserved in liquid nitrogen. The cryo preserved pollen after thawing retained its viability as seen by *in vitro* germination and pollination.

The *in vitro* gene bank was enriched with new varieties and cultivars of black pepper, cardamom, turmeric, ginger and vanilla and 700 genotypes are being maintained. Genomic DNA was isolated from nutmeg and 150 accessions of black pepper and 100 accessions of cardamom were added to DNA bank.

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

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

Compilation of information was completed in 33 spices namely, black pepper, ginger, cardamom, turmeric, large cardamom, vanilla, long pepper, nutmeg, cinnamon, cassia, bay leaf, aniseed, star anise, angelica, caper, greater galangal, horse radish, hyssop, basil, rosemary, parsley, saffron, sage, sweet flag, tarragon, caraway, black cumin, ajowan, coriander, cumin, fennel, fenugreek, dill and celery.

The compiled information contains details on taxonomy including valid name, classification, synonyms, common names and other biological details, namely, habit and habitat, diagnostic features, reproductive biology, breeding system, major diseases and pests, economic potential and uses.

4. DBT: On-farm evaluation of tissue culture derived plants of black pepper

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

Multiplication of primary cultures

Five hundred *in vitro* cultures of black pepper varieties, Sreekara and Subhkara, were established for supply to Biotechnology and Model Floriculture Centre, Thiruvananthapuram, and Spices Board, Kochi, for further multiplication and field evaluation.

Multiplication of biocontrol agents

Glomus fasciculatum, an efficient strain of VAM for growth promotion and suppression of root pathogens such as, *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita* was multiplied on graminaceous hosts (*Zea mays*) for application during transfer of plants to the soil. Another efficient fungal biocontrol agent, *Trichoderma harzianum*, was multiplied on sorghum and on liquid medium (molasses) and mixed in sterilized decomposed coir pith.

Fig. 10. PCR amplified DNA from turmeric rhizomes

Field evaluation

A yield evaluation trial with micropropagated plants was laid out at Calicut with 170 plants. *T. harzianum* was applied at the time of planting and the establishment was good.

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

(B. Sasikumar and T. John Zachariah)

A simple and rapid method for isolating good quality DNA from matured rhizome tissues of turmeric and ginger, which are rich in polysaccharides, polyphenols and alkaloids was standardized. The isolated DNA proved amenable to PCR amplification and restriction dissection.

DNA was isolated from leaf tissues of 200 turmeric accessions and 81 ginger accessions for characterization of ginger and turmeric germplasm by RAPD (Fig. 10). Sixty random decamer primers were screened during the study among which 34 gave good, clear consistent bands.

Final Reports

1. NATP: Molecular characterization and preparation of molecular maps in black pepper

(K. Nirmal Babu, M. Anandaraj, Johnson K. George, B. Sasikumar and P. N. Ravindran)

Objectives

- Molecular profiling of important *Piper* species and cultivars of black pepper to study their interrelationships and genetic diversity.

- Development of mapping populations of black pepper and preparation of preliminary molecular maps.

Molecular profiling of germplsm

RAPD profiles of Piper *species*

RAPD profiles of *Piper* species were developed using polymorphic primers namely, OPA-09, OPA-15, OPB-07, OPC-07, OPC-13, OPD-02, OPD-03, OPE-11, OPF-09 and OPF-14. The *Piper* species used for the study included *P. longum, P. hapnium, P. mullesua, P. attenuatum, P. argyrophyllum, P. hymenophyllum, P. babbudani, P. trichostachyon, P. galeatum, P. sugandhi, P. psuedonigrum, P. nigrum, P. schmidtii, P. wightii, P. barberi, P. betle, P. chaba-1, P. chaba-2, P. magnificum, P. colubrinum-1, P. colubrinum-2, P. arboreum* and *P. ornatum*. The RAPD profile data was analysed by NTSYS software to study the interrelationships between the species. The analysis indicated that *P. attenuatum and P. argyrophyllum* were close to each other. *P. pseudonigrum* was closest to *P. nigrum* followed by *P. galeatum. P. schmidti, P. wightii* and *P. hymenophyllum* were farthest to *P. nigrum* followed by *P. colubrinum. P. caliba* was in a distinct group separated both from *P. nigrum* and *P. longum*.

RAPD profiles of black pepper cultivars and varieties

RAPD profiles of 14 major cultivars namely, Karimunda, Kottandan, Balancotta, Neelamundi, Kuthiravally, Kalluvally, Arakulam munda, Narayakodi, Thomankodi, Perambramunda, Poonjaranmunda, Valiakaniakadan, Cheriyakaniakadan and Uthirancotta, and 10 released varieties namely, Panniyur-1, Panniyur-2, Panniyur-3, Panniyur-4, Panniyur-5, Sreekara, Subhakara, Panchami, Pournami and Palode-2 were developed using 30 polymorphic primers. The interrelationships between the cultivars/varieties were studied utilizing the RAPD profile data. The analysis showed the similarity between Subakara and Sreekara. Palode-2, a selection from Kottanadan-2, showed similarity to Kottanadan, and Panniyur-3 with both Valiakaniakadan and Cheriyakaniakadan. However, Panniyur-1 was clearly placed in a separate group.

RAPD profiles of Phytophthora *tolerant and susceptible cultivars*

RAPD profiles of 11 each of *Phytophthora* susceptible and tolerant cultivars were developed using polymorphic primers namely, OPA-03, OPA-09, OPA-134, OPA-18, OPC-02, OPA-07, OPA-18; OPD-03; OPE-11, OPF-14 and OPF-09 and their interrelationships were studied. The study indicated that, in general, the tolerant lines formed a cluster of their own. However, no specific marker was found consistently associated with *Phytophthora* tolerance.

Development of mapping population

Two mapping populations were developed using Panniyur-1 and Subhakara (Karimunda) and P-24 (tolerant to *Phytophthora*) and Subhakara. Out of 1000 hybrids, 218 of the first cross were transferred to the field. As the percentage of fruit set was very low in P-24, few hybrid seeds were produced and around 20 hybrids of P-24 x Karimunda were transferred to polybags. Two hundred selfed progenies of parents namely, Panniyur-1, Subhakara and P-24 were established in order to study the residual heterozygosity of parents. Data on segregation of important qualitative characters like shoot tip colour, leaf shape, base and tip, root variation, growth and branching pattern of the progenies were recorded.

RAPD and AFLP profiles of mapping population

DNA was isolated form 100 hybrid progenies of Panniyur-1 x Subhakara using modified method of Ausubel *et al.* (1995) and RAPD profiling was done using 40 random primers. Data was collected on about 150 loci for preparing molecular maps. Work is in progress as part of the institute project to collect data on another 300 loci before the preliminary map is prepared. A protocol for developing AFLP in black pepper was standardized.

2. DBT: Field evaluation of tissue cultured plants of spices and assessment of their genetic stability using molecular markers

(K. Nirmal Babu, E. V. Soniya*, B. Sasikumar and P. N. Ravindran)

*Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram

Objectives

- Evaluation of field performance of tissue cultured plants of major spices.
- Estimation of genetic fidelity of tissue cultured plants using molecular markers.
- Development of micro satellite markers in black pepper.

Black pepper

Field evaluation and morphological characterization of micropropagated black pepper plants were conducted with 1000 plants at 4 locations. The morphological data (on 28 characters) was collected from 2, 3 and 4 year old plants separately. Analysis of data indicated that there was no significant difference among the micropropagated and conventionally propagated plants. The micropropagated plants flowered early in the case of *cv.* Karimunda and *cv.* Aimpiriyan showed vigorous growth. Early flowering in 3-year old plants can be attributed to the residual effect of BA used for multiplication of black pepper in *in vitro* and the vigorous growth to the branching habit of micropropagated plants.

Standardization of RAPD protocols in black pepper was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, and IISR, Calicut, and 70 operon primers were tested for random amplification in two genotypes namely, Panniyur-1 and Karimunda. Genetic stability of 20 randomly selected micropropagated plants of two released varieties namely, Panchami and Subhakara was analysed. RAPD profiles showed uniformity in the micropropagated plants except in one or two genotypes.

Development of micro satellite markers

Simple sequence repeats for micro satellite detection in black pepper was identified at RGCB. Micro satellite (CA and TC) enriched libraries of *Piper nigrum* genomic fragments were constructed in p GEM –T vector using the method adopted by White and Powel (1997). Clones of CA repeats were analysed for their sites through plasmid isolation and PCR amplification using SP6 and T7 universal primers. The size of the inserts were determined which ranged from 118 bp to 850 bp. A few of the clones were sequenced and the primers were designed from the sequenced information. These primers were used to screen a few genotypes in the black pepper germplasm to detect polymorphism.

Ginger

Field evaluation of 300 plants developed

Fig. 11. *In vitro* developed microrhizomes of ginger

through micropropagation and 500 plants of microrhizhome-derived plants (*vars.* Jamaica and Australia) was conducted at Calicut (Fig. 11). *In vitro* developed microrhizomes with a fresh weight of 5–10 g were directly planted in the field without hardening with 70% establishment. Data was collected for morphological characters such as number of tillers, plant height, leaf number, leaf length, leaf breadth, internodal distance and petiole length. Microrhizome-derived plants although slow in field establishment, were on par with control plants within 2–3 months. Microrhizome-derived plants were observed to produce more number of tillers per plant than the micropropagated and control plants. The microrhizomes gave a fresh rhizome yield of 100–800 g per plant and with an average yield of 10 kg/bed (3 m^2) of fresh ginger. Plants developed from microrhizomes were more stable than micropropagated plants. Genetic fidelity analysis indicated variations in micropropagated plants even without callus phase.

Turmeric

Field evaluation of micropropagated and microrhizhome-derived plants (500) was conducted at Calicut. Data was collected for morphological characters such as number of tillers, plant height, leaf number, leaf length, leaf breadth, inter nodal distance and petiole length. Analysis of data indicated that there was no significant variation between micropropagated and control plants. Microrhizomes of turmeric with a fresh weight of 2–8 g were directly planted in the field, as in the case of ginger, without hardening with 90% establishment. The microrhizomes gave a fresh rhizome yield of 320–750 g per plant and with an average yield of 14 kg/bed (3 m^2) of fresh turmeric. The results of genetic stability analysis were similar to that of ginger. The RAPD profiles of microrhizome-derived plants did not show any noticeable polymorphism. The banding pattern indicated that microrhizhome-derived plants were more stable when compared to micropropagated plants.

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, Johnson K. George, K. V. Saji, T. John Zachariah, K. S. Krishnamurthy, S. Devasahayam, Santhosh J. Eapen and M. Anandaraj)

Evaluation of new lines

Coll. 1041, a clone of black pepper *cv.* Thevanmundi continued to exhibit disease tolerance at Valparai (3000 ft MSL) (Tamil Nadu) in the collaborative trials with Tata Tea Ltd. (Fig. 12). Cumulative mean yield (row trial) for 7 years at Valparai indicated the superiority of Coll. 1041, HP-105 and HP-813 over control Panniyur-1 (Table 15). These lines performed superior to Panniyur-1 in large block trials (blocks of 20–55 plants) also. OPKm, Coll. 1041, HP-780 and HP-1411 performed better at Peruvannamuzhi (Table 16).

Fig. 12. Collection 1041, a promising line of black pepper

Table 15. Evaluation of promising black pepper lines at Valparai

Line/Control	Mean fresh yield/vine (kg)*	Bi	s^2bi	Remarks
Coll. 1041	5.17	1.59	1.38**	Tolerant to disease
HP-105	6.40	0.92	1.96**	-
HP-813	2.78	1.05	0.76**	High oleoresin
Panniyur-1 (Control)	1.08	1.61	0.26**	-

* Mean of 7 years; ** $P < 0.01$

Table 16. Evaluation of promising black pepper lines at Peruvannamuzhi

Line/Control	Mean fresh yield/vine (kg)	Dry recovery (%)	Remarks
OPKm	2.29	32.0	-
Coll. 1041	2.32	31.2	-
HP-1411	2.87	30.4	-
HP-780	2.69	39.5	Early bearing and high dry recovery
Sreekara (Control)	2.16	32.8	-

The promising lines planted in farmer's plots in four northern districts of Kerala through Technology Mission on Black Pepper was also monitored. Data on vine height, number of spikes, number of berries/spike, number of laterals/vine and yield per vine were recorded.

Evaluation of promising hybrids/lines

A few of the hybrids/open pollinated (OP) lines identified during 2001–02 based on first year's yield data (3rd year of planting) continued to yield better (Table 17).

Table 17. Evaluation of promising black pepper hybrids at Peruvannamuzhi*

Hybrid/Control	Mean fresh yield/vine (kg)
HP-127	1.00
HP-1262	1.36
HP-1264	0.90
HP-1293	0.90
OP Neelamundi	1.36

* First year data

Evaluation of cv. *Neelamundi*

Seventeen clonal progenies of black pepper and seven OP lines of black pepper *cv.* Neelamundi were evaluated for yield. The yield of the clonal progenies ranged from 0.50 kg (Acc. 1446) to 1.36 kg (Acc. 4116). As compared to clonal lines, OP progenies showed more variation in yield and the yield per vine ranged from 0.081 kg to 1.360 kg/vine.

Intervarietal crosses

Sixty-seven intervarietal cross combinations and 13 interspecific combinations were attempted in black pepper and the number of seeds harvested varied from 10 to 140 per cross.

2. Breeding cardamom for high yield and resistance to *katte* disease

(*D. Prasath and M. N. Venugopal*)

Evaluation of hybrids and selections

Growth and yield parameters were recorded in 56 diallel hybrids. Based on *per se* performance, heterosis and specific combining ability over 3 years, 15 hybrid combinations were short-listed for yield and susceptibility to leaf blight. The short-listed hybrid combinations were planted for clonal multiplication.

Standard heterosis for yield ranged from –23.22 to 150.33 and 25 hybrids recorded significant positive heterosis. Wherever *katte* resistant lines were used as female parents in the hybrids, there was positive relationship between seedling vigour (nursery) and yield (main field). However, a similar relationship did not exist in other parental combinations.

Evaluation of open pollinated seedlings

Evaluation of open pollinated progenies of 14 high yielding and *katte* resistant accessions led to the identification of 9 plants with high yield and resistance to leaf blight.

Molecular characterization

DNA was isolated from a number of Malabar, Mysore, Vazhukka types and other accessions with distinct morphological characters. However, initial studies of RAPD showed very little polymorphism.

Comparative evaluation trials

Two new comparitive yield trials (CYTs) were laid out comprising of 19 and 16 short-listed hybrids, selections and compound panicle types. These treatments are being compared with promising released, pre released and popular varieties (Green Gold) (Tables 18 and 19).

Table 18. Evaluation of promising hybrids of cardamom in clonal multiplication block

Hybrid	No. of tillers/ plant	Increase over Green Gold (%)
Hybrid-1	97	7.7
Hybrid-2	102	13.3
Hybrid-3	123	36.7
Hybrid-5	106	28.9
NKE-27 x NKE-12	154	71.1
NKE-27 x RR-1	100	11.1
NKE-34 x CCS-1	110	22.2
MB-3 x RR-1	115	27.8
OP (NKE-11)	155	72.2
Green Gold	90	-

Medium with BAP 1 mg/l + kinetin 1 mg/l + cephotaxime 250 mg/l + kanamycin 50 mg/l) for selection and regeneration. Plantlets could be successfully regenerated from black pepper leaf explants treated with *Agrobacterium* strain containing osmotin construct.

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

(Johnson K. George and B. Sasikumar)

DNA was isolated from 12 accessions of *Piper* including 8 accessions of cultivated black pepper for determination of parental polymorphism. The RAPD analysis was done to identify male parent specific RAPD bands for identification of true hybrids in crosses involving cultivated and wild germplasm. The male parent specific band OPE 01_{600} in *P. nigrum* (wild) was useful in identification of true hybrids in the population derived from the cross with cultivated black pepper.

Differential display RT- PCR was done with total RNA isolated from leaves of black pepper plants (*var.* Sreekara) having spikes with developing berries and juvenile plants for identification of genes involved in quality attributes. Two cDNA fragments (300 bp

Table 19. Performance of parental lines and their F_1 hybrids in cardamom

Character	Range			No. of hybrids showing desirable heterosis
	Per se performance	Heterosis (%)		
	Parents	Crosses (F_1)	Standard heterosis	
Plant height (cm)	140.56–175.00	85.00–203.67	-51.54–16.38	7
Tillers/plant	18.67–25.57	10.00–34.33	-61.17–33.33	17
Bearing tillers/plant	7.47–10.50	4.42–15.80	-60.44–50.00	26
Panicles/plant	18.00–39.00	8.67–61.33	-70.11–111.49	24
Yield/plant	203.70– 629.00	41.00–1954.00	-93.48–210.65	20

3. Biotechnological approaches for crop improvement in black pepper

(K. Nirmal Babu, K. V. Saji and M. Anandaraj)

Genetic transformation

Various variables such as co-cultivation period, preculture of explants, temperature and pH were tested for genetic transformation and co-cultivation with *Agrobacterium* for 48 h was found to be best. Leaf explants from both juvenile and mature tissues and zygotic embryos were used as explants for transformation.

The *Agrobacterium* treated tissues were cultured in the selection medium (Woody Plant approx.) corresponding to differentially expressed genes were tagged using two random 13 mer primers in second strand synthesis.

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

(R. Ramakrishnan Nair and Johnson K. George)

Embryogenic cultures of *cv.* Subhakara were maintained and plants were regenerated. Cytological analysis of 40 regenerated plants indicated normal chromosome number of 2n=52.

Four random operon primers namely, OPD-16, OPD-3, OPE-02 and OPF-05 were used to develop RAPD profiles of somatic embryo-derived plants of black pepper *cv.* Subhakara in comparison with the mother plant. Profiles generated by operon primers OPD-16, OPE-02 and OPD-03 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-05 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 4 of the regenerated plants.

6. Cytogenetics and reproductive biology of major spices

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

Chromosome indexing was done in 10 accessions of ginger and 16 accessions of turmeric. All the ginger accessions analysed had a chromosome number of 2n=22. In turmeric, 14 accessions had 2n=63, one (Acc. 30) had 2n=61 and another (Acc. 768) had 2n=80.

Studies on floral biology of ginger indicated that the flowers opened between 2.45 pm to 3.00 pm and anthesis occurred 15 min after flower opening. The flowers dropped by next day morning. Self-pollination of 309 flowers from 32 accessions of ginger did not result in seed set.

Seeds from fruits of different developmental stages of *Curcuma longa* were histologically analysed to study the embryo developmental stages. Six different developmental stages starting from early globular to cotyledonary stages were identified. The cotyledonary embryo resembled that of cardamom.

7. Rootstock-scion interactions in tree spices

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

Nutmeg

Standardization of grafting

Seedlings of *Myristica malabarica*, *M. beddomeii* and *M. fragrans* were raised in the nursery for grafting. Preliminary trials on softwood grafting were carried out with two different scions namely, A9-4 and A9-69 on *K. andamanica* and a success of 28% and 36%, respectively was achieved. Softwood grafting of nutmeg with scions from three different rootstocks namely, *M. fragrans*, *M. malabarica* and *M. beddomeii*, was carried out and success was highest on *M. fragrans* (90%) followed by *M. malabarica* (76%) and *M. beddomeii* (68%).

Evaluation of grafts for productivity

The grafts on three different rootstocks using two different scions namely, A9-4 and A9-69 were planted in the field at Peruvannamuzhi for yield evaluation. Morphological observations such as, height, girth and number of branches were recorded on the grafts and it was observed that growth was highest on *M. beddomeii* rootstocks for both A9-4 and A9-69 scions, followed by *M. malabarica* and *M. fragrans*. In general, it was observed that the growth of grafts with A9-69 scions was superior to that of A9-4. Field trials for evaluating the grafts are also in progress in farmer's plots at Kolli Hills and Pollachi in Tamil Nadu.

Evaluation of grafts for drought

The grafts on three different rootstocks using two different scions namely, A9-4 and A9-69 were planted for in the field at Peruvannamuzhi for evaluation for drought tolerance.

Budding

Budding was carried on three different rootstocks of *Myristica* sp. namely, *M. fragrans*, *M. malabarica* and *M. beddomeii* and only 30% success was obtained (Fig. 13).

Clove

Evaluation of grafts of clove on *Syzygium heynianum*, *S. cuminii* and *S. aromaticum* indicated that they are performing well (growth) in the field at Peruvannamuzhi.

Fig. 13. Patch budding in nutmeg

Final Reports

1. *In vitro* selection for resistance to soft rot and bacterial wilt in ginger

(*K. Nirmal Babu, M. J. Ratnambal, M. S. K. Shetty, G. N. Dake, T. G. Nageswara Rao, A. Kumar and N. K. Leela*)

Objectives

- Development of callus and cell culture systems in ginger to produce somaclonal variations.
- Evaluation of regenerated plants for somaclonal variation.
- Isolation of pathotoxin from *Pseudomonas* spp. and *Pythium* spp.
- Screening of somaclones against rhizome rot and bacterial wilt.
- *In vitro* selection against pathogens at culture level to obtain disease resistant mutants.

Micropropagation and direct plant regeneration

Ginger plants could be successfully regenerated from vegetative bud explants with about 10 multiple shoots, when they were cultured on Murashige and Skoog (MS) medium supplemented with 1 mg/l NAA. The shoots developed roots in the same medium. This system could be used for production of disease-free material.

Indirect plant regeneration

Callus was induced from vegetative bud, leaf and ovary explants on MS medium supplemented with 2 mg/l 2,4-D. Plants could be regenerated from all the explants tried on MS medium supplemented with 10 mg/l BAP and 0.2 mg/l 2,4-D. Regeneration was by organogenesis and embryogenesis and in some cases both were seen in the same culture. Plant regeneration from ovary derived callus was the most efficient for plant recovery (over 150 plants per culture tube) followed by calli derived from leaf, anther and vegetative buds. This high regeneration system was ideal for large scale production of somaclones and for *in vitro* selection to develop tolerant lines to biotic and abiotic stresses.

In vitro culture of floral buds

When immature inflorescences of ginger were cultured on MS medium supplemented with 10 mg/l BAP and 0.2 mg/l 2,4-D, development of plantlets was noticed in 38% of the cultures by the conversion of floral buds into vegetative buds. But when individual flowers were separated and cultured on the same medium, the ovary developed into a tri-locular capsule and subsequently plantlets could be recovered from the fruits probably due to chance *in vitro* pollination.

Plant regeneration from anther derived callus culture

Anthers at the uninucleate microspore stage, after cold treatment (0°C) for 7 days, cultured on MS medium supplemented with 2.0 mg/l 2,4-D, developed profuse callus. Plantlets could be regenerated from this calli on MS media supplemented with 6.0 mg/l BAP and 0.2 mg/l 2,4-D.

Isolation of protoplasts

Protoplasts could be successfully isolated from leaf mesophyll tissue collected from *in vitro* grown plantlets. The enzyme combination was macerozyme R10 (0.5%) + onozuka cellulase R10 (3%). The protoplast yield was 3.5 x 10^4 ml and were viable and could be successfully plated on culture media and made to develop up to microcalli stage. Successful isolation and culture of protoplast is an important step in gene transfer protocols and also for somatic hybridization.

Cell suspension cultures

Cell suspension cultures were successfully established when callus was cultured in liquid medium supplemented with 0.5 mg/l 2,4-D and kept on gyratory shakers at 80 rpm. The cell suspensions were maintained for over 2 years by repeated subcultures. A few oil cells were noticed among these suspension cultures.

Isolation of DNA

DNA could be successfully isolated from sprouting buds and leaf tissues of ginger using CTAB method. The amount of DNA was estimated to be 120 µg/ml. The DNA was free of RNA and protein and was of good quality.

Transient expression of GUS in embryogenic callus

Transient expression of β-glucuronidase gene was successfully induced when ginger embryogenic calli were bombarded with microprojectiles coated with plasmid based on pUC 19 vector, using Biolistics Particle Delivery System. This vector has two chimeric genes, ubiquitin-β-glucuronidase (*Ubi-GUS*) and ubiquitin-phosphinothricin acetyl transferase (*Ubi-bar*) as selectable markers. High level of GUS expression was noticed in the bombarded calli.

Field establishment and evaluation

The micropropagated plantlets could be established in soil with 80% success, when they were planted in a potting mixture of garden soil, sand and vermiculite in equal proportion and kept in humid chamber for 30 days. Subsequently, they were transferred to pots and were evaluated for various morphological characters. The micropropagated plants performed like seedlings of similar Zingiberaceous crops and only very small rhizomes (1 cm in diameter) were formed during the first year. The size of the rhizomes increased over the years and developed into normal size comparable to that of mother plants after the third year. This indicates that tissue cultured plants cannot be directly used for commercial cultivation and need at least three crop seasons in the nursery or field before they develop rhizomes of normal size suitable for commercial planting.

Variations in somaclones

Variations were observed in somaclones produced directly as well as through intervening callus phase for plant height, number of tillers, rhizome yield, rhizome size, etc. (Fig. 14). Biochemical evaluation indicated variability in important quality attributes like dry recovery, oleoresin and essential oil. RAPD profiles of the 'variant somaclones' showed polymorphism indicating variability.

Isolation of crude toxin from **Ralstonia solanacearum**

The crude toxin produced by *R. solanacearum*, the causal organism of bacterial wilt disease was isolated and tested for its bioassay with cut shoots of ginger. This crude toxin was effective even after sterilization at 120°C for 15 min and can be added in the culture medium before sterilization. The crude toxin was incorporated in the culture medium at various concentrations and embryogenic cultures were cultured in the medium containing toxin. The surviving plantlets were transferred to the field for further screening. Though reduced growth rate was noticed, the concentration of toxin had no deleterious effect on the culture.

Fig. 14. Variations in rhizomes of ginger somaclones

Extraction of culture filtrate from **Pythium aphanideramatum**

Culture filtrate (CF) was extracted from *P. aphanideramatum*, the causual organism of rhizome rot disease and was used on callus and embryoid cultures at various concentrations. Though CF extracted on second day appeared more effective in reducing the growth rate, the concentration of CF was insufficient to have any significant effect.

In vitro screening for bacterial wilt

An easy *in vitro* screening technique was developed by directly inoculating the bacterial wilt pathogen *R. solanacearum* in cultures of small plantlets maintained aseptically in culture bottles. The disease reaction could be noticed in 15 days where the susceptible plants showed typical yellowing symptoms of bacterial wilt.

Screening for reaction to diseases

About 1000 somaclones established in polybags were screened for their reaction to *P. aphanidermatum* and *R. solanacearum* infections by direct application of the pathogen to the collar region of the young shoots. The disease development was manifested as yellowing and curling of leaves with water soaked patches at the collar region.

Twenty-two somaclones were planted (in both greenhouse and field) in endemic areas of *R. solanacearum* Biovar III. All the plants succumbed to the disease after 120 days of inoculation.

Eleven somaclones, which escaped infection to *P. aphanideramatum* in the polybag screening were found to be susceptible after 120 days of inoculation in a pot culture experiment with virulent strain of the pathogen when blended mycelial mats were used as inoculum.

Identification of promising lines

A few promising lines (with regard to yield, and other quality attributes) were identified from the somaclones. Lines MP-61-9, MP-69-3, CR-67 and CR-1222 were promising with yields of 870 g, 685 g, 536 g and 431 g of fresh rhizome per plant, respectively. Line CR-1222 had bold rhizomes. When these somaclones were indexed for their susceptibility to *P. aphanidermatum* by direct inoculation of the pathogen, seven lines namely, MP-61.9, MP-70, MP-72, MP-76, CR-10, CR-816 and CR-822 though susceptible survived the first cycle of inoculation, indicating a very low level of tolerance. Among them, MP-61-9, CR-10-1, CR-816 and CR-822 yielded 870 g, 367 g, 373 g and 358 g fresh rhizome per plant, respectively. MP 74-15 and CR-1222 were high yielders with 780 g and 600 g of fresh rhizomes per plant respectively. MP-74-15 with a fresh rhizome yield of 780 g and CR-818 with a fresh rhizome yield of 398 g also had bold rhizomes. In addition, the rhizomes of CR-1222 were attractive with extra bold fingers. Based on biochemical assays, CR-818 (3.6%), MP-49-7, MP-70-4, MP-97 and CR-10-1 (all with 3.8%) were identified as low fibre types.

Though a high degree of somaclonal variation was found in ginger with regard to various morphplogical and quality characters, and a few promising lines were identified, no significant variation could be found with regard to resistance to *P. aphanidermatum* and *R. solanacearum*.

Externally Funded Projects

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)

Genetic transformation in black pepper

Leaf explants from juvenile and mature tissues and zygotic embryos were used as explants for transformation. The explants were co-cultured with *Agrobacterium* strain GV 2260 containing osmotin for 48 h. The treated tissues were in the selection medium (Woody Plant's Medium with BAP 1 mg/l + kinetin 1 mg/l + cephotaxime 250 mg/l + kanamycin 50 mg/l). Four plantlets were regenerated from leaf discs treated with *Agrobacterium* containing osmotin.

Isolation of disease resistant gene candidates

Amplification of chitinase and glucanase genes

Amplification of chitinase gene from *Piper colubrinum* was achieved using chitinase specific primer designed at the institute. Chitinase sequences (both protein and nucleic acid) were obtained from SWISS-PROT and TrEMBL (Protein Knowledge Base) in ExPASy (Expert Protein Analysis System) Molecular Biology Server. Barley chitinase structure was taken as a model to the design primers. Sequence homologues was obtained by FASTA search in PDB. The chitinase sequences were subjected to BLAST search. The chitinase sequences were then submitted for multiple alignment programme provided by the online software CLUSTAL-W by Genebee Software.

The total RNA isolated from *P. colubrinum* (control and challenged with *P. capsici*) were used to study gene specific amplification. First strand cDNA synthesis was done with oligo d (T) 15 mer primer using standardized methods. Second strand (chitinase specific) amplification was done by a chitinase gene specific 15 mer primer. A 600 bp band was found to be differentially amplified from RNA sample of *P. colubrinum* challenged with *P. capsici*. The conserved amino acid sequence SHETTGG was used to design degenerate primers using primer Premier 5 Software. A similar approach was followed for designing primers for amplification of α-1, 3 glucanase from *P. colubrinum*. Experiments are in progress to amplify α-1, 3 glucanase specific gene fragments using 15 mer primer designed based on partially conserved amino acids of glucanase genes.

Amplification of genes from P. colubrinum

Late blight resistance protein identified in *Solanum* species was used to identify sequence homologues from public database. Multiple alignment was done using CLUSTAL-W software. Two 15 mer primers were designed based on partially conserved amino acid sequences of these disease resistance genes. Multiple bands were amplified in RNA samples from *P. colubrinum* challenged with *P. capsici*. Sequencing of the cDNA fragments are to be done for further confirmation.

Differential display amplification from mRNA

First strand synthesis of mRNA isolated from *P. colubrinum* leaves (control and challenged with *P. capsici*) was done using oligo d (T) 15 mer primers. Second strand amplification was performed using 10 mer operon primers OPE-1 (5'CCCAAGGTCC3') and OPE-8 (5'TCACCACGGT3') and a differentially expressed band of approximately 500 bp was amplified in the sample from challenged plant.

A similar experiment done with 13 mer random primer sequence (5'AAGCTTCGGCATA3') resulted in differential amplification of more than 1000 bp size in challenged plant sample in comparison with total RNA sample from control plant.

Isolation of disease resistant gene

Differential display RT-PCR was carried out using RNAs isolated from *P. colubrinum* (resistant to *Phytophthora*) to tag genes expressed in *P. colubrinum* in response to *Phytophthora* inoculation. The cDNAs were resolved in agarose gel. Two cDNA bands corresponding to differentially expressed genes in *P. colubrinum* were eluted and reamplified for cloning and sequencing. DDRT-PCR was done with total RNA isolated from *P. colubrinum* leaves challenged with *Phytophthora capcisi* and normal leaves from uninoculated plants as control (Fig. 15). Gene specific primers were designed for amplification of disease resistance and disease response genes. cDNAs corresponding to a putative chitinase gene (~ 600 bp) and disease resistance gene (2 cDNAs of ~ 250 bp) was amplified using one chitinase specific primer.

Molecular characterization of cardamom

RAPD profiles of related species

RAPD profiles were developed in 13 related genera/species of *Elettaria* (*E. cardamomum*, *Amomum subulatum*, *A. ghaticum*, *A. microstephanum*, *A. melegueta*, *A. involucratum*, *Alpinia purpureae*, *Al. galanga*,

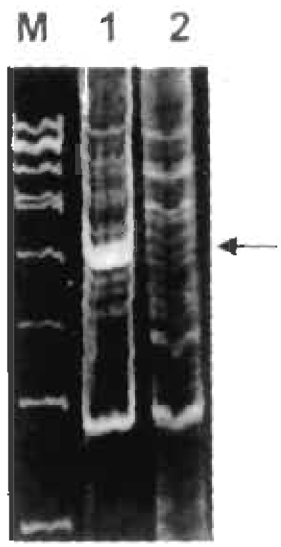

Fig. 15. Amplification of chitinase specific c DNA from *Piper colubrinum*
(M. Maker, 1. Amplification from plant sample challenged with *Phytophthora capsici*, 2. Amplification with RNA sample from *Piper coubrinum* (control) (Arrow indicates a 600 bp cDNA band in '1')

Al. mutica and *Hedychium coronarium*.) with 25 primers. All the primers showed high polymorphism. *Al. galanga* was found to be nearest to cardamom while the farthest was *A. ghaticum* and *A. microcarpum*, among the genera studied.

RAPD profiles of released varieties and promising lines

RAPD profiles were developed, using 25 random primers, in 24 promising genotypes of cardamom, namely, ICRI-1, ICRI-2, RR-1, NKE-9, NKE-12, NKE-19, CCS-1, PV-1, PV-2, Mudigere-1, Mudigere-2, Sampaji Clone, MCC lines 12, 21, 40, 85 and 346, S-1, PS-27, SKP-165, SKP-170, MHC-26, MHC-27 and Hybrid-3. Most of the RAPD profiles developed had monomorphic pattern between genotypes and only a few polymorphic bands were detected. A pattern unique to SKP-170 and MCC-40 could be detected with primer OPF-5 and OPB-13, respectively. Multi Branch Sterile differed from other 95 genotypes in the absence of a distinct band at 1 kb region, whereas Mini Pink could be distinguished from rest of the genotypes with an absence of a prominent band at 1.4 kb region with the primer, OPC-6.

RAPD markers linked to *katte* disease resistance

RAPD profiles were developed using 8 primers in 22 Malabar types (11 each resistant and susceptible to cardamom mosaic virus). Though consistent markers associated with *katte* resistance could not be identified, a few RAPD loci seem to be associated with resistant genotypes.

III. Mega project: System approach for sustainable production of spices

(Project leader: K. Kandiannan)

1. Nutritional requirement of improved varieties of spices

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

Black pepper

Evaluation of micronutrients

The effect of foliar application of Zn on yield and quality of black pepper was evaluated at farmer's plots in comparison with soil application of Zn. The number of laterals produced was marginally higher in Zn treated plots. But the mean yield recorded at all the locations were similar to no Zn application except at Kasaragod where Zn application recorded higher yields (Table 20). The quality (oleoresin content) of black pepper berries was also on par among treatments.

Effect of Mg nutrition

The effect of Mg @ 0, 50, 75 and 100 kg/ha was studied in black pepper. Soil availability of Mg increased with application of different doses of Mg fertilizers. The available K was on par among levels of Mg fertilizers added along with uniform application of N, P and K. No clear influence on yield and chlorophyll content was observed by application of Mg fertilizers in all the three varieties studied, since the yields were very low.

Nutrient requirement for targeted production

Field experiments were laid out at Madikeri by fixing the targets of production of black pepper at three levels namely, 7.5, 10.0 and 14.0 kg/vine. Soil samples were collected from plots with different yield targets and based on their initial soil test value the N, P and K requirement for the fixed targets were applied. At the first target level of 7.5 kg/vine, the recorded yield varied from 4.1 to 11.0 kg/vine. Under the target level of 10 kg/vine, the recorded yield varied from 5.0 to 9.2 kg/vine and under the target level of 14 kg/vine, it varied from 6.3 to 14.8 kg/vine and the deviation from the targets (7.5, 10 and 14 kg) were 11.1%, 32.4% and 39.9%, respectively (Table 21).

Ginger and turmeric

Evaluation of micronutrients

The effect of application of Zn at different levels in combination with or without coir compost was studied in ginger and turmeric for the second year. Soil availability of Zn and P were estimated at harvest. The available P content was low due to higher rate of P fixation. Soil Zn availability increased sig-

Table 20. Effect of zinc application in black pepper

Location	No. of laterals/0.25m²			Fresh yield (kg/vine)		
	Control	S Zn	F Zn	Control	S Zn	F Zn
Peruvannamuzhi	20.0	29.0	25.0	1.44	0.80	1.26
Kasaragod	22.0	25.0	23.0	1.53	2.69	2.05
Kannur	15.0	12.0	15.0	2.22	2.74	2.66
Kozhikode	6.1	6.3	6.8	2.34	2.24	2.05
Wyanad	6.9	9.0	8.3	1.15	1.02	1.06

S Zn = Soil zinc; F Zn = Foliar zinc

Table 21. Fertilizers recommended and yield recorded under different yield targets in black pepper

Fertilizer (g/vine)			Targeted fresh yield (kg/vine)	Actual fresh yield (kg/vine)	Deviation from target (%)
N	P	K			
156	20	-	7.5	6.7	11.1
258	104	-	10.0	6.4	32.4
378	107	53	14.0	8.1	39.9

nificantly with levels of Zn applied. Addition of coir compost did not influence soil Zn and P availability. Leaf Zn level also followed the pattern of levels of Zn applied and foliar treatment recorded the highest concentration.

Fresh yield of ginger rhizomes was highest in Zn application @ 5 kg/ha; all the other levels were on par with control and foliar application of Zn. Fresh yield of turmeric was on par among treatments (Table 22 and 23).

Table 22. Effect of Zinc and coir compost on zinc availability and yield of ginger

Zn (kg/ha)	Soil Zn (mg/kg)			Yield (kg/3m^2 bed)		
	Without CC	With CC	Mean	Without CC	With CC	Mean
0	0.9	1.0	1.0	9.1	9.1	9.1
5.0	1.7	2.1	1.9	12.7	11.9	12.3
7.5	3.8	3.6	3.7	11.8	7.9	9.8
10.0	8.3	7.4	7.8	7.8	9.0	8.4
15.0	14.4	16.0	15.2	7.5	8.2	7.9
Foliar	2.3	1.9	2.1	9.0	7.9	8.5
Mean	5.2	5.3		9.7	9.0	
CD (P<0.05) for Zn levels		1.4			1.4	
CD (P<0.05) for CC		NS			NS	
CD (P<0.05) for Zn x CC		1.9			1.9	

CC = Coir compost

Table 23. Effect of zinc and coir compost on soil and leaf zinc availability and yield in turmeric

Zn (kg/ha)	Soil Zn			Leaf Zn			Yield (kg/3m^2 bed)		
	Without CC	With CC	Mean	Without CC	With CC	Mean	Without CC	With CC	Mean
0	0.74	0.67	0.70	14	12	13	9.8	13.2	11.5
5.0	1.23	2.23	1.73	20	15	18	10.9	12.4	11.7
7.5	1.99	2.40	2.44	27	20	24	10.9	11.7	11.3
10.0	2.98	3.64	3.31	40	24	32	10.4	11.4	10.9
15.0	6.67	6.13	6.40	42	41	41	11.4	13.0	12.2
Foliar	1.67	1.67	1.67	45	40	43	11.0	11.3	11.1
Mean	2.55	2.87	-	31	25	-	10.7	13.2	-
CD (P<0.05) for Zn levels		0.68			5.0			NS	
CD (P<0.05) for CC		NS			2.9			1.2	
CD (P<0.05) for Zn x CC		0.96			7.1			2.9	

CC = Coir compost

Organic farming

Trials on organic cultivation of ginger and turmeric were initiated to develop a package for organic farming. The beds were solarized and the biocontrol agent *Trichoderma harzianum* was applied to control rhizome rot. Organic inputs like 10 kg FYM, 1 kg neem cake, 2 kg vermicompost, 0.5 kg ash and 150 g rock phosphate were applied per bed of 3 m^2 and top dressed with 2.5 kg of enriched coir pith compost and vermicompost. The incidence of rhizome rot disease was very high in ginger and was controlled by periodical removing of infected plants along with infested rhizomes. The yield recorded was low with an average of 8 kg/bed in ginger and 10 kg/bed in turmeric.

IPNM schedules

An IPNM experiment to scale down fertilizer use was laid out with sources like neem cake (NC) and phosphate solubilizing bacteria (PSB) to supplement the nutrients. The soil K, Ca and Mg availability significantly increased with application of neem cake, phosphobacteria and P as rock phosphate.

The increased level of P application up to 2 P dosage and PSB application did not increase the available P in the soil beyond 3.7 mg/kg, probably due to higher degree of P fixation in the soil. The lower pH (5.1 to 5.3) in the soil probably did not support phosphobacterial dissolution during the 9 month duration of the crop.

In turmeric, a higher rhizome yield of 12.8 kg/bed was recorded in plots applied with higher dose of P along with half the recommended dose of N with NC and FYM + PSB. However the rhizome yield was on par among the treatments (Table 24).

In ginger, significantly high rhizome yield of 10.9 kg/bed was recorded in the treatment where FYM, neem cake, PSB and rock phosphate were applied along with half the recommended dose of urea, which was on par with recommended NPK. The oleoresin content was also highest (4.73%) in this treatment (Table 25).

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 analysed for nutrients and *Azospirillum* population before and after imposing treatments. Initial nutrient status of soil was deficient in available phospho-

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

Treatment	pH	N	P	K	Ca	Mg	Zn	Yield
		(mg/kg)						(kg/3m^2 bed)
T1	4.80	60	3.30	173	241	112	0.57	10.250
T2	5.10	67	3.70	204	215	158	0.71	11.830
T3	5.20	69	2.50	227	328	164	0.69	12.850
T4	5.30	67	2.80	197	334	175	0.83	12.930
CD ($P<0.05$)	0.07	55	0.97	49	28	19	0.12	NS

T_1 = Recommended package of practice (POP); T_2 = Half N & P of POP + Phosphate solubilizing bacteria (PSB) + Neem cake (NC); T_3 = Half N of POP + PSB + NC; T_4 = Twice POP + PSB + NC

Table 25. Effect of IPNM on soil nutrient availability and yield of ginger

Treatment	pH	Yield (kg/3m² bed)	N	P	K	Ca	Mg	Zn
			(mg/kg)					
T1	5.07	10.970	103	0.62	59	219	56	0.93
T2	5.29	8.380	107	1.70	83	320	90	1.03
T3	5.49	10.940	108	2.30	97	366	101	1.05
T4	5.39	9.720	98	1.70	71	274	75	0.88
CD (P<0.05)	0.02	2.500	7	0.72	32	85	22	NS

T_1 = Recommended package of practice (POP); T_2 = Half N & P of POP + Phosphate solubilizing bacteria (PSB) + Neem cake (NC); T_3 = Half N of POP + PSB + NC; T_4 = Twice POP + PSB + NC

rous and high in nitrogen and potash. Pre treatment *Azospirillum* population was low. *Azospirillum* population gradually increased and highest *Azospirillum* counts were recorded in the treatment where inorganic nitrogen 50% + Mg were applied. Nitrogen and phosphorous contents were more in plots in which *Azospirillum* was applied but no significant difference was observed. Potassium availability increased significantly in plots in which *Azospirillum* was applied.

3. Biometeorological investigations and modelling in black pepper

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

Weather is one of the key components that controls agricultural production and in some areas as much as 80% of the variability in yield is due to variability in weather conditions. This project was initiated with the objectives of surveying and summarizing the existing knowledge and data on the effect of meteorological factors on black pepper yield, making use of meteorological information in order to relate meteorological elements to yield response of black pepper and studying the quantitative dependence of black pepper yield on meteorological variables. The available published information on the role of climate/weather on production of black pepper was collected for further analysis.

Final Reports

1. Investigation on spices based cropping systems

(V. S. Korikanthimath, A. K. Sadanandan, K. Sivaraman, M. N. Venugopal, Rajendra Hegde, S. J. Ankegowda, C. K. Thankamani, K. Kandiannan and V. Srinivasan)

Objectives

- Compatibility of various crop combinations in spices based cropping systems.
- Organic recycling of crop residues and farm wastes in the system.
- Impact of crop combinations on VAM and other associated micro organisms.
- Minimizing cost of cultivation by sharing common cultural operations.
- Generating gainful employment to small and marginal farmers and agricultural labourers, round the year.
- Building up of congenial microclimate with various crop combinations.
- Economic analysis.

Soil nutrient status

On-farm trials on mixed cropping of cardamom under two agroecological cropping situations namely, Robusta coffee in Kodagu and arecanut in Uttar Kannada districts (Karnataka) were monitored. Soil samples were collected at two depths (0–15 cm and 16–30 cm) in cardamom based cropping system and analysed for pH, organic carbon, major and micronutrients. The soils were acidic and the pH ranged from 5.0 to 6.1; in general the surface soil had higher pH than the sub-surface layer. The soil was high in organic carbon content which was maximum in coffee (3.3%) followed by cardamom (3.0%) in mixed cropping of cardamom + coffee combination. Interestingly lowest organic carbon content of 1.3% was noticed in sole crop of coffee. P content was higher in nutmeg (22.6 ppm) cultivated in combination with cardamom, while lowest P content was observed in cardamom (3.2 ppm) cultivated along with cinnamon. The highest K content (498 ppm) was noticed in clove cultivated with cardamom, while lowest K content (105 ppm) was observed in cardamom grown with black pepper.

Ca and Mg contents in surface soil were higher in cardamom as sole crop than coffee alone. However, highest Ca content (1238 ppm) was observed in coffee grown with cardamom followed by nutmeg (967 ppm), allspice (920 ppm) and cardamom (900 ppm) with clove, which were higher than sole crop of cardamom. The highest Fe content (26.8 ppm) was observed in clove and allspice grown in combination with cardamom and lowest was with black pepper (14.3 ppm). Allspice, cinnamon and clove recorded higher content of Mn than the sole crop of cardamom. In general, Zn and Cu availability in soil was not influenced by cardamom based cropping system. However, slightly higher availability of Zn was observed with clove (1.0 ppm) and allspice (0.9 ppm) than sole crop of coffee. In general, Cu content was higher in mixed cropping system than sole crop of cardamom. Nutrient availability was generally higher at surface than subsurface layer and availability of major and micronutrients was higher in cardamom based cropping system than sole crop of cardamom and coffee.

Leaf nutrient content

Leaf N (3.50%) and P contents (0.21%) were highest in coffee mix cropped with cardamom. Cardamom in combination with black pepper as well as mono crop recorded highest K (3%) content. Allspice leaves contained highest Ca content (3.4%) whereas Mg was maximum in nutmeg leaves (0.42%). Fe content was maximum (237 ppm) in coffee mix cropped with cardamom. Mn content was highest (805 ppm) in cardamom mix cropped with coffee. Zn content was highest in nutmeg mix cropped with cardamom. It was interesting to note that Cu content was highest (119.9 ppm) in black pepper mix cropped with cardamom.

Microclimate and physiological parameters

In all the crop combinations, humidity inside the leaf chamber was lower than inside the crop canopy. Crop combinations involving cardamom + cinnamon and cardamom + black pepper allowed better infiltration to photosynthetically active radiation compared to other combinations. Among the various crop combinations, transpiration rate was highest in cinnamon and lowest in clove and cardamom as sole crop (Table 26). Leaf temperature was highest when coffee and cardamom were grown as sole crops. Highest stomatal conductance was recorded in black pepper when grown along with cardamom. Allspice recorded highest photosynthetic rate. Intercellular CO_2 concentration was highest in coffee as mixed crop.

Table 26. Physiological parameters in cardamom based cropping systems

Crop combination	Transpiration (µmole H_2Om^{-2})	Leaf temperature (°C)	Stomatal conductance (µmol CO_2m^{-2})	Photosynthesis (µmol CO_2m^{-2})	Inter cellular CO_2 conc.(µmol CO_2m^{-2})
Nutmeg + Cardamom	0.8	31.6	0.03	3.30	88.4
Clove + Cardamom	0.4	33.2	0.01	0.91	179.9
Allspice + Cardamom	1.0	32.4	0.04	6.80	178.0
Cinnamon + Cardamom	2.1	32.6	0.10	4.33	174.3
Black pepper + Cardamom	1.4	31.7	1.30	4.58	157.5
Coffee + Cardamom	0.9	31.9	0.04	2.86	216.2
Coffee (sole crop)	1.8	34.7	0.06	2.70	187.0
Cardamom (sole crop)	0.4	33.4	0.01	2.05	79.8

Light distribution

Light availability in cardamom based cropping systems was studied by measuring photosynthetically active radiation (PAR) in open, below shade trees, above and below cardamom plants. PAR in open condition ranged from 1296.0 to 1559.7 µmol $m^{-2}sec^{-1}$. The light available to component crops ranged from 64.5 to 610.2 µmol $m^{-2}sec^{-1}$. Light intercepted by shade trees ranged from 58.0% to 97.5%. The leaf area index of shade trees ranged from 0.84 to 2.66, which indicated the need for shade regulation. Light received by cardamom in various cropping systems ranged from 54.2 to 906.4 µmol $m^{-2}sec^{-1}$ with a mean of 265.92 µmol $m^{-2}sec^{-1}$. The available light, which filtered through cardamom canopy and which reached nearer to ground ranged from 15.00 to 111.35 µmol $m^{-2}sec^{-1}$ with a mean of 63.94 µmol $m^{-2}sec^{-1}$. Light intercepted by cardamom canopy ranged from 28.0% to 89.5% and leaf area index of cardamom ranged from 0.65 to 2.08.

Rhizosphere microflora

Initial soil samples were collected at three locations and at two horizontal zones from plant base (0–15 and 16–30 cm) for studying the microbial load (Table 27). The mycorrhizal spore count was 21.33/100 cc of soil.

The populations of commonly occurring fungi, bacteria and actinomycetes were also enumerated in the rhizospheres of component crops under study. Nitrogen fixers, phospahte solubilizing bacteria (PSB) and phosphate solubilizing fungi population were also analysed. Bacterial population in the rhizosphere of cardamom under mixed crop was lower compared to its sole crop but higher than the control plot (uncultivated check). However, under mixed crop situations, cardamom rhizosphere contained consistently higher populations of fungi, phosphate solubilizers and nitrogen fixers when compared to sole crop and control plot. Actinomycetes population did not show much variation in various systems.

Table 27. Initial microbial population (other than VAM) in the soil

Depth	Bacteria ($\times 10^5$)	Fungi ($\times 10^3$)	Actinomycetes ($\times 10^5$)	N_2 fixers ($\times 10^3$)	P-solubilisers ($\times 10^4$)
1–15 cm	18.35	14.10	5.56	8.71	5.93
15–30 cm	9.97	7.18	3.81	16.77	43.73

Disease incidence

The incidence of various diseases of main and component crops was recorded during mid and post-monsoon periods. Mosaic infection in cardamom occured randomly in only two blocks depending on access to inoculum source and viruliferous aphids. Leaf blight of cardamom, anthracnose of black pepper and leaf spot of clove and cinnamon caused by *Colletotrichum gleosporiodes* were also observed.

Growth and yield parameters

Among the various crop combinations, cardamom + clove recorded maximum ground coverage (9200.4 m^2), leaving only 799.6 m^2 of the ground area uncovered. Least ground coverage was observed in crop combination with cardamom and coffee (6552.3 m^2). Cardamom exhibited better growth in terms of its height in combination with allspice, whereas its growth was reduced in combination with black pepper. Sole crop of cardamom recorded maximum number of bearing tillers and total tillers. Among the mixed crop treatments, cardamom in combination with clove showed better tillering (Table 28).

All the component crops (coffee, black pepper, clove and cinnamon) except nutmeg have commenced yielding (Table 29).

2. Management efficiency of whole farm approach in farming-a study on spices based farming system

(V. S. Korikanthimath, Rajendra Hegde, S. J. Ankegowda and P. Rajeev)

Objective

The project was initiated to study the management efficacy of whole farm approach in spices based farming systems.

Pilot study

A pilot study was conducted by interviewing a group of farmers, adopting rapid rural appraisal technique, to decide the choice of enterprise to be included in the system. The study indicated that, there was a system of integrated farming earlier and cattle were considered an important component of it. As the area under various plantation crops was less, the available forest land and common village land served the purpose of grazing for animals. However, with the expansion of area under plantation crops later, the area available for animals to graze gradually disappeared. With the availability of ready to use chemical fertilizers, the system changed to specialized farming of plantation crops. The planters obtained adequate quantity of manures from neighboring districts at a cheaper cost earlier. But

Table 28. Growth characters of cardamom in various cropping systems

Crop combination	Height (cm)	No. of tillers		
		Bearing	Non-bearing	Total
Cardamom + Nutmeg	169	21.0	14.3	35.3
Cardamom + Clove	172	26.7	19.3	46.0
Cardamom + Allspice	174	18.9	16.7	35.6
Cardamom + Cinnamon	167	19.2	16.8	36.0
Cardamom + Black pepper	154	15.2	11.3	26.5
Cardamom (sole crop)	165	25.3	19.2	44.5

Table 29. Yield of cardamom in various cropping systems

Crop combination	Yield (kg/ha)								
	1993	1994	1995	1996	1997	1998	1999	2000	Average
Cardamom + Nutmeg	1066	292	498	831	346	584	492	510	577
Cardamom + Clove	1138	271	521	240	512	840	817	830	646
Cardamom + Allspice	1031	304	477	226	454	744	709	726	584
Cardamom + Cinnamon	853	273	368	163	179	294	328	338	350
Cardamom + Black pepper	770	147	336	181	297	487	402	410	379
Average of cardamom in crop combinations	972	257	440	208	358	590	550	518	487
Cardamom (sole crop)	1235	398	593	346	657	912	833	842	727

in recent times, extensive cultivation of ginger in the neighbouring districts and expansion of area under various plantation crops have made organic manures a scarce commodity. In Uttara Kannada and parts of Shimoga where a majority of the planters are small holders, integrated system of cultivating arecanut, cardamom, banana, paddy, coconut and animal husbandary make farming viable and sustainable. Hence in Kodagu District also, the need for an integrated whole farm approach is being felt by many progressive farmers.

Component crops

Various component crops such as, cardamom, arecanut, banana, *Garcinia gummigutta, Artocarpus lakoocha,* black pepper, coffee and vanilla along with bee keeping were included in the whole farm farming system.

Biomass production and recycling

Biomass production was assessed in various cropping systems. Co-1 grass produced an average of 67.33 t/ha per year biomass, which was used as mulch and also sold as fodder. Biomass production in coffee and cardamom gardens were 13.88 and 6.94 t/ha per year. In freshly planted cardamom gardens, sunhemp (*Crotalaria juncea*) and dhaincha (*Sesbania aculeata*) were sown separately in between rows of cardamom and 14 t/ha of fresh biomass (3.7 t/ha of dry matter) was harvested in 50 days and utilized for composting.

Growth parameters

Various growth parameters of component crops were measured (Table 30).

Table 30. Growth parameters of component crops

Crop	Height (m)	No. of branches/ leaves/ tillers
Robusta coffee	1.00	9.5
Arabica coffee	1.00	10.9
Garcinia	3.00	24.8
Allspice	3.67	31.0
Arecanut	3.08	6.4
Cardamom	1.56	12.3

Micro climate

Air temperature and relative humidity in canopy area of component crops were recorded (Table 31).

Light transmission

Photosynthetically active radiation received in open conditions, below the shade tree

Table 31. Microclimatic conditions in component crops

Crop	Air temperature (°C)	Relative humidity (%)
Robusta coffee	29.8	56.2
Arabica coffee	29.4	55.0
Garcinia	29.8	54.0
Allspice	29.8	55.2
Arecanut	30.4	55.6
Cardamom	30.4	52.0

canopy, above and below component crops, light transmission/interception and leaf area index of shade trees and component crops were determined (Table 32). The canopies of shade trees permitted better light transmission in coffee plots. In other crops maximum amount of light was intercepted by shade trees itself indicating the need for shade regulation.

Table 32. Light availability to component crops

Crops	PAR (μmol $m^{-2}sec^{-1}$)							
	Open	Below shade tree	Transmission (%)	Above crop	Below crop	Transmission (%)	Shade tree	Crop
Arabica Coffee	1160	470	40.5	189	168	89.0	1.4	0.3
Robusta Coffee	1127	470	41.7	262	102	38.9	1.5	1.9
Arecanut	1157	30	2.6	44	30	68.2	4.9	0.7
Banana	1158	34	2.9	44	13	29.6	4.7	3.3
Garcinia	1157	91	7.9	91	30	33.0	4.9	1.0

3. Vermicompost production using organic wastes available in cardamom areas

(V. S. Korikanthimath, Rajendra Hegde and S. J. Ankegowda)

Objective

The project was undertaken to study the efficacy of vermicomposting in comparison with traditional methods of composting of organic wastes in cardamom growing areas and to study the influence of vermicompost on soil properties and growth and yield performance of cardamom.

Vermicomposting

During the first year of study (1994–95), a species of local earthworm collected from Sirsi (Uttara Kannada), Sagar (Shimoga) and Appangala in Karnataka and an introduced African earthworm species (*Eudrillus eugenia*) were multiplied on a mixture of shade tree leaf litter and cowdung (10:1). The initial observations revealed that the introduced earthworm was efficient in conversion of organic wastes and its multiplication rate was also considerably high when compared to the local earthworm. But the local earthworm could not survive in the conditions at which *E. eugenia* performed very well. Studies on composting of wastes initiated during 1994–95 indicated that coffee husk alone was not a good food for earthworm (Table 33) and its population decreased in this medium. When various organic wastes were mixed with leaf litter, the composting rate increased along with earthworm population. The composting rate increased substantially when all the wastes were mixed in equal proportion with cowdung slurry.

The nutrient (P, K, Mg and Ca) and organic carbon contents of the cardamom rhizo-

Table 33. Vermicomposting of organic wastes

Waste	Conversion (%) *	Weight gain of worms (g)**
Coffee husk	22.6	-31.7
Coir dust	56.2	200.0
Arecanut husk	30.8	135.0
Leaf litter	41.3	211.0
Coir dust + Leaf litter	46.3	100.0
Areca husk + Leaf litter	37.6	300.0
Coffee husk + Leaf litter	20.1	25.0
Coir dust + Areca husk + Coffee husk + Leaf litter	80.7	150.0

* After 3 months; ** Initial worm wt. = 100 g

sphere soil almost doubled when it was supplied with vermicompost or farm yard manure (FYM) for two seasons. The pH of soil changed towards neutrality. However, the contents of most nutrients were only marginally higher in vermicompost compared to normal compost (Table 34).

Table 34. Nutrient content of vermicompost and compost from organic residues of cardamom plantation

Nutrient	Vermicompost	Compost
N (%)	1.2	1.1
P (%)	0.5	0.2
K (%)	0.1	0.4
Ca (%)	0.9	0.8
Fe (ppm)	4179.0	4119.0
Mn (ppm)	401.0	400.0
Zn (ppm)	78.0	78.0
Cu (ppm)	56.0	50.0
S (%)	0.9	0.5

Growth and yield of cardamom

Growth parameters

The effect of vermicompost on growth of cardamom was studied in comparison with neem cake, FYM and NPK applied in various combinations (Table 35).

Table 35. Details of treatments in the experiment to study the effect of vermicompost on cardamom

T1	Control
T2	NPK (50:50:100 g/plant in two splits)
T3	FYM (10 kg/plant in two splits)
T4	Neem cake (NC) (1kg/plant in two splits)
T5	Vermicompost (VC) (3 kg/plant in two splits)
T6	NPK + NC
T7	NPK + VC
T8	NPK + VC
T9	Half VC+ Half NPK
T10	Half VC + Half NC + Half NPK
T11	Half FYM + Half NC
T12	Half FYM + Half NC+ Half NPK

Observations on number of tillers per plant, number of leaves per tiller and plant height were recorded at 6 months after treatment (Table 36). There was no significant difference among the treatments in number of tillers per plant and plant height; however there was significant difference between the

Table 36. Effect of vermicompost on growth of cardamom (6 months after treatment)

Treatment	Height (cm)	No. of tillers/ plant	No. of leaves/ tiller
T1	130.6	16.1	36.0
T2	135.0	20.8	36.9
T3	127.8	18.3	35.1
T4	136.1	21.6	37.6
T5	133.9	16.9	37.7
T6	123.3	20.1	33.2
T7	127.2	21.7	34.3
T8	138.3	20.8	35.8
T9	126.1	18.0	35.8
T10	137.8	21.9	37.7
T11	124.6	15.2	36.4
T12	137.2	19.3	37.4
CD ($P<0.05$)	NS	NS	3.9

Refer Table 35 for details of treatments

treatments in number of leaves per tiller. The number of leaves per tiller was higher (37.7) in treatments which received vermicompost alone (T_5) and 50% V C + 50% N C + 50% NPK (T_{10}), which was significantly higher than T_6 (100% NPK + FYM), which recorded lowest number of leaves per tiller (33.2).

Observations on plant height, number of tillers, number of bearing tillers, number of non-bearing tillers and fresh yield recorded during 2001 indicated that there was no significant difference between treatments for growth and yield characters (Table 37).

Nutrient content in soils

The application of organic and inorganic fertilizers significantly influenced nitrogen and phosphorus contents in the soil. However, there was no significant difference among the treatments on the K content of soil. The highest contents of 241.67 kg/ha of N, 88.93 kg/ha of P and 737.67 kg/ha of K were observed in the treatments T_8, T_6 and T_6, respectively. The lowest contents of N (135.33 kg/ha), P (14.33 kg/ha) and K (165.00 kg/ha) were observed in the treatments T_9, T_1 (control) and T_5, respectively. Relatively higher build up of nutrients in soil was observed when the recommended dose of fertilizer + organic fertilizers were applied.

Secondary micronutrients

The highest contents of Ca^{2+} (585.33 ppm), Mg^{2+} (204.67 ppm), Fe (43.67 ppm), Mn (43.00 ppm), Zn (2.6 ppm) and Cu (7.93 ppm) were observed in the treatments T_{11}, T_3, T_9, T_6 and T_3, respectively. The lowest contents of Zn (1.67 ppm) and Fe (27.67ppm) were observed in control while that of Ca^{2+} (404.33 ppm) and Mg^{2+} (111.0) in T_{10}, Mn (14.33) in T_5 and Cu (4.50 ppm) in T_8 (Table 38).

The secondary micronutrient content (except Mn) in soil was not significantly influenced by application of organic and inorganic fertilizers.

Microbial population

Bacterial population ranged from 140.19 x 10^5 to 274.93 x 10^5 in various treatments with

Table 37. Effect of vermicompost on growth and yield of cardamom

Treatment	Plant height (cm)	No. of tillers	No. of bearing tillers	No. of non-bearing tillers	Fresh yield (g/plant)
T1	166.0	22.9	13.9	9.1	129.1
T2	150.3	21.9	12.5	9.3	114.7
T3	165.7	22.7	13.4	9.3	59.1
T4	205.3	34.0	18.5	15.6	230.3
T5	174.7	23.0	13.7	9.8	410.8
T6	169.0	21.4	10.4	10.3	78.0
T7	176.0	26.4	15.1	11.5	176.7
T8	155.7	18.9	9.8	9.1	64.7
T9	170.0	25.0	14.5	11.2	106.8
T10	192.0	30.2	18.6	11.6	194.3
T11	193.3	20.1	17.6	9.8	134.9
T12	183.7	26.1	16.9	12.2	181.4
CD (P<0.05)	NS	NS	NS	NS	NS

Refer Table 35 for details of treatments

Table 38. Effect of vermicompost on micronutrient content in soil (after 6 months)

Treatment	Ca^{2+}	Mg^{2+}	Fe	Mn	Zn	Cu
			(ppm)			
T1	512.00	121.00	27.67	17.00	1.67	5.03
T2	460.00	121.00	42.67	29.00	2.10	7.13
T3	498.67	204.67	43.67	23.67	2.17	7.93
T4	553.67	199.00	38.33	23.00	2.10	6.33
T5	449.00	112.00	32.67	24.33	2.07	6.43
T6	425.00	136.33	38.03	22.00	2.60	5.37
T7	427.67	129.67	09.33	30.67	2.23	5.50
T8	437.67	120.33	31.33	15.67	2.20	4.50
T9	542.67	542.67	138.33	43.00	2.10	4.57
T10	404.33	111.00	32.00	21.17	2.43	5.47
T11	585.33	189.33	40.00	21.33	2.57	6.80
T12	418.33	158.67	35.67	24.67	2.17	6.23
CD ($P<0.05$)	NS	NS	NS	24.63	NS	NS

Refer Table 35 for details of treatments

a mean of 195.63 x 10^5. Fungal population ranged from 58.41 x 10^4 to 135.70 x 10^4 with a mean of 80.21 x 10^4. Actinomycetes ranged from 28.46 x 10^3 to 76.26 x 10^3 with a mean of 43.78 x 10^3. *Beijerinckia* population ranged from 15.33 to 71.64 with a mean of 37.7 (Table 39).

Light interception

Light availability (PAR) in various treatments was studied. Light availability in the plot was 1520.78 µmol $m^{-2}sec^{-1}$. Almost 70.7% of light was intercepted by shade trees and the mean leaf area index of shade trees was approximately 1.4. Cardamom crop

Table 39. Effect of vermicompost on microbial population in soil (1 year after planting)

Treatment	Bacteria (x 10^5)	Fungi (x 10^4)	Actinomycetes (x 10^3)	*Beijerinckia* (x 10^2)
T1	186.88	97.55	45.84	43.45
T2	209.55	72.18	36.09	36.09
T3	195.92	66.87	43.01	35.20
T4	183.45	86.47	42.07	18.70
T5	150.94	62.50	57.78	15.33
T6	274.93	83.19	76.26	71.64
T7	209.13	93.87	35.65	57.03
T8	140.19	58.41	40.89	58.41
T9	153.85	69.82	36.69	50.89
T10	211.75	135.70	31.59	19.89
T11	219.23	68.44	51.04	20.87
T12	211.79	67.59	28.46	24.90
Mean	195.63	80.21	43.78	37.70

Refer Table 35 for details of treatments

received a mean of 335.36 μmol $m^{-2}sec^{-1}$ light (PAR) and intercepted 62.9%.

Externally Funded Projects

1. NATP: 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*)

Four field experiments namely, assessment of soil and water conservation measures in cardamom; systems of planting for efficient utilization of rainwater in cardamom; assessment of frequency and duration of irrigation in cardamom and assessment of soil and water conservation in coffee based multi-storeyed cropping system were laid out (Fig. 16). Soil and water conservation treatments were imposed during 2001–02. Soil properties and nutrient status were recorded during initiation of experiments. Data on growth of main crop and vegetative barriers were recorded at six monthly intervals.

The trials indicated that contour staggered trenches of 2.00 m x 0.30 m x 0.45 m in alternate rows of cardamom and coffee prevented water and soil loss by restricting soil movement and also improved soil moisture status. The contour staggered trenches along with vegetative barriers like pineapple, french bean and ginger also restricted soil and water loss. Though ginger performed well during the first year of planting, the incidence of diseases was higher during the subsequent years. Pineapple and french bean performed well when grown between two rows of cardamom or coffee. In the experiment on irrigation schedules, drip irrigation and sprinkler irrigation treatments were better than control in term of growth parameters. A farm pond was constructed which would be sufficient to meet the water requirement of the whole farm in future.

Fig. 16. Intercropping of pineapple in cardamom plantation for soil conservation

2. Government of Kerala: Technology mission on black pepper

(*V. Srinivasan, B. Sasikumar, C. K. Thankamani, S. S. Veena and Y. R. Sarma*)

The research experiments under Technology Mission on Black Pepper were initiated in 2000 in farmers fields in northern districts of Kerala with the objective of identifying and popularizing high yielding varieties, evolving technologies for sustainable production and developing cost effective technology for management of diseases of black pepper.

Evaluation of varieties

Under identification of location specific varieties programme at Kasaragod and Kozhikode districts, the new black pepper lines and varieties/hybrids from Pepper Research Station, Panniyur (P-1 to P-5) performed superior to local varieties as well as Sreekara, Subhakara, Panchami and Pournami in terms of plant height, number of laterals and spikes produced. However, among the new lines, OPKm was superior in terms of growth and earliness in bearing. In Wyanad District, the new lines, Panniyur varieties and Sreekara performed well (Fig. 17).

Evaluation of organic nutrition

In the experiment on effect of organics on yield and quality of black pepper, the treatment farm yard manure (FYM) + neem cake (NC) + phosphobacteria (PB) recorded maximum yield (2.32 kg/vine) and was on par with all other treatments except check. Application of biofertilizers (*Azospirillum* and phosphobacteria) along with FYM or with half the recommended N and P doses helped in increasing soil fertility and yield. Under the experiment on standardization of low input practices for black pepper, the yield was maximum (2.24 kg/vine) in standard package of practices (POP) treatment that was on par with ½ POP + Zn, ½ POP + BF and ½ POP + NC.

Evaluation of biocontrol agents

In the experiment on adoption of biocontrol schedules for checking *Phytophthora* diseases in the nursery, the efficacy of fungicides namely, potassium phosphonate and metalaxyl alone and in combination with the biocontrol agent (BCA) *Trichoderma harzianum* was tested in comparison with the standard practice of drenching copper oxychloride and spraying Bordeaux mixture. BCA along with chemicals had positive effect on growth parameters and disease suppression. Under field conditions also, yellowing was least in BCA and potassium phosphonate treatments.

Evaluation of propagation methods

Under standardization of rapid method of generation of planting materials of high yielding varieties, the performance of conventional method of propagation was evaluated in farmer's plots at Kannur and Kozhikode districts. At Kannur, the success rate varied from 68% to 70% whereas, at Kozhikode it was 78%. Among the varieties, maximum success was obtained in Panniyur-2 at both the locations.

Fig. 17. Evaluation of promising lines of black pepper in farmer's field

3. ICAR Emeritus Scientist Scheme: Integrated plant nutrient management strategy for breaking black pepper yield plateau and quality upgradation

(*A. K. Sadanandan*)

Objectives

The objectives of the project was to study the impact of Integrated Plant Nutrient Management System (IPNS) on improving soil and crop quality and breaking the yield plateau in black pepper.

Introduction

Studies on effect of IPNS in increasing nutrient availability in soil, crop uptake of nutrients, yield and quality improvement in black pepper was taken up for two years (2000–02). The greenhouse experiments/ field trials were conducted in four major black pepper growing agro-ecological situations in Calicut, Wyanad (Kerala), Pollibetta (Karnataka) and lower Palanis

(Tamil Nadu). The treatments comprised of application of soil conditioners, different sources of plant nutrients such as organic, inorganic (including micronutrients) and biofertilizers in balanced proportion.

Nutrient availability and yield response

Liming black pepper growing acid soils @ half the lime requirement of the soil enhanced dehydrogenase enzyme activity, microbial population and nutrient availability in soil and crop productivity. Application of inorganic N at half the recommended dose (50 kg N/ha) and the balance N (50 kg N/ha) as organic form (FYM and de-oiled neem/groundnut cakes) in conjunction with biofertilizers (*Azospirillum* and phosphobacteria) each @ 10.9 cfu per vine and micronutrients (zinc, boron and molybdenum @ 5, 2, 1 kg/ha) enhanced spiking intensity, nutrient uptake, yield and berry volume of black pepper. Significant increase in leaf N, for individual years as well as over the years, was also noticed due to adoption of IPNS. The bulk density of the soil decreased due to the adoption of IPNS. FYM application significantly decreased the bulk density irrespective of the soils.

Soil quality

Improving and maintaining soil quality provided economic benefits in increasing productivity. Among the soil quality attributes, organic carbon and cation exchange capacity (CEC) were the most discriminating attributes in all areas. The soil organic matter and CEC contributed substantially to the ability of the soil to accept, hold, and release nutrients to black pepper vines at Wyanad, Pollibetta and lower Palanis. Adoption of IPNS decreased *Phytophthora* disease incidence to around 2% over the years in all areas.

Crop quality

Piperine and oleoresin contents of black pepper significantly increased due to adoption of IPNS. At Calicut and Pollibetta, maximum piperine and oleoresin contents were recorded when inorganic N was applied at half the recommended dose and the balance N as organic form supplemented with biofertilizers and micronutrients. However, in Wyanad and lower Palanis, maximum piperine and oleoresin contents were observed when FYM and inorganic fertilizers were supplemented with micronutrients.

Yield targeting

To attain a targeted yield of 3 kg/vine from the soils of Wyanad, the nutrient requirement based on soil test value, yield and nutrient uptake of the berry, was N: P_2O_5: K_2O @ 128:206:136 kg/ha. For attaining a targeted yield of 8 kg/vine for the soil of Pollibetta, the N: P_2O : K_2O requirement was 151:60:212 kg/ha.

Economics

The overall beneficial effect of IPNS was reflected in cost-benefit analysis. For every rupee invested, the benefit was Rs 5.59 at Wyanad, Rs 6.76 at lower Palanis and Rs 16.70 at Pollibetta.

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)

PAL activity and curcumin levels

The activity of phenyl alanine lyase (PAL) was studied in 20 accessions of turmeric with low (>2%) and high (<5%) levels of curcumin to study the relationship between PAL activity and curcumin levels (Tables 40 and 41). There was a direct correlation between PAL activity and curcumin levels, which confirmed its role as the rate-limiting enzyme.

Table 40. Phenyl alanine lyase activity in high curcumin accessions of turmeric

Acc./Var.	Curcumin (%)	PAL activity (μM trans-cinnamic acid released/ min/mg protein x 10^{-2})
Alleppey	7.0	105.4
Suguna	6.5	58.9
Sudarsana	6.8	79.9
Acc. 38	7.5	20.0
Acc. 126	7.2	76.0
Acc. 290	8.5	66.7
Acc. 325	7.1	21.3
Acc. 329	7.5	49.0
Acc. 356	7.6	26.6
Acc. 583	7.0	28.2
Acc. 585	7.0	72.6

Tracer studies with ^{14}C-CO_2

One month old turmeric plants were exposed to ^{14}C-CO_2 and samples were taken at 24, 48, 72 and 96 h intervals and afterwards at monthly intervals to study the incorporation of the labeled compound, using liquid scintillation counter. Incorporation of ^{14}C was maximum in leaves up to 96 h, which was then translocated to the roots, which indicated that roots were the probable site for later stages of curcumin biosynthesis.

Table 41. Phenyl alanine lyase activity in low curcumin accessions of turmeric

Acc.	Curcumin (%)	PAL activity (μM trans-cinnamic acid released/ min/mg protein x 10^{-2})
Acc. 64	2.7	6.1
Acc. 88	3.8	7.4
Acc. 257	3.3	4.6
Acc. 303	2.8	15.3
Acc. 310	2.4	16.7
Acc. 335	3.4	11.9
Acc. 367	3.0	6.5
Acc. 656	3.1	5.9
Acc. 672	2.9	8.3
Acc. 673	2.5	1.1

2. Characterization of drought tolerance in black pepper

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

One hundred black pepper germplasm lines were screened for drought tolerance and a few relatively tolerant accessions (HP-23, HP-29, HP-301 and HP-328) were identified which were further screened under potted conditions.

Relative water content and membrane damage

The tolerant and susceptible accessions had similar values for relative water content and membrane damage up to 5 days after stress induction (DASI), but the differences were more pronounced at 10 DASI.

Ascorbate peroxidase activity

Ascorbate peroxidase activity increased under stress condition and varied among the hybrids. The increase was pronounced after 5 DASI but at 10 DASI, the activity started declining and reached below control (without water stress) levels (Table 42).

Table 42. Ascorbate peroxidase activity in black pepper hybrids

Hybrid	Control (without stress)	With stress	
		5 DASI	10 DASI
HP-23	3.9	5.7	3.3
HP-29	3.5	5.0	2.9
HP-37	3.0	4.9	3.0
HP-42	3.6	4.7	2.6
HP-58	4.0	5.0	3.2
HP-109	3.7	4.3	3.1
HP-192	3 4	4.8	2.7
HP-301	3.6	5.0	3.2
HP-303	3.9	5.1	3.1
HP-328	4.2	5.8	3.7
CD (P<0.05)	NS	0.6	0.4

Values indicate units/mg protein; DASI=Days after stress induction

Table 43. Superoxide dismutase activity in black pepper hybrids

Hybrid	Control (without stress)	With stress	
		5 DASI	10 DASI
HP-23	5.1	5.0	5.0
HP-29	6.2	6.5	5.9
HP-37	4.7	5.2	5.0
HP-42	5.0	5.3	4.7
HP-58	5.4	5.6	5.2
HP-109	5.4	5.9	5.1
HP-192	5.3	5.1	4.8
HP-301	5.7	6.0	5.2
HP-303	5.6	5.4	4.9
HP-328	4.9	4.7	4.6
CD (P<0.05)	NS	NS	NS

Values indicate units/mg protein; DASI=Days after stress induction

Superoxide dismutase activity

Superoxide dismutase activity did not alter much due to stress and the differences among the hybrids was not significant. The increase in activity was minimal and was more or less stable even after 10 DASI (Table 43).

Glutathione reductase activity

Glutathione reductase activity also increased during water stress and significant differences were noticed among the hybrids. But after 10 DASI, the activity reached back to control (without stress) levels (Table 44).

Table 44. Glutathione reductase activity in black pepper hybrids

Hybrid	Control (without stress)	With stress	
		5 DASI	10 DASI
23	23.0	31.0	24.0
29	19.0	24.0	21.0
37	20.0	26.0	21.0
42	16.0	21.0	18.0
58	19.0	23.0	19.0
109	21.0	27.0	22.0
192	22.0	26.0	22.0
301	19.0	26.0	21.0
303	16.0	20.0	16.0
328	18.0	24.0	20.0
CD (P<0.05)	1.5	2.4	1.9

Values indicate units/mg protein; DASI=Days after stress induction

3. Characterization of drought tolerance in cardamom

(*S. J. Ankegowda, K. S. Krishnamurthy and D. Prasath*)

Evaluation of cardamom accessions

Six accessions namely, APG-18, APG-34, APG-149, DR-3, DR-6 and DR-16 were grown in cement pots under rain-out shelter for one year and moisture stress treatment was imposed by withholding irrigation. Growth parameters like plant height, number of leaves and number of tillers were recorded at initiation and at end of stress. Soil moisture content was also recorded at different intervals.

Growth characters

Growth parameters at initial stress period did not show significant variation. Genotypic variation for plant height was significant at the end of stress (Table 45); DR-16 recorded maximum plant height and APG-34 recorded minimum plant height. Significant variations were also recorded for number of leaves, number of dry leaves and number of tillers per clump. APG-18 recorded maximum number of leaves followed by APG-149. Number of dry leaves was more in DR-16 followed by DR-6. Number of tillers was more in DR-16 and APG-18.

Root characteristics

Root length, total number of roots and root weight were significantly reduced under stress treatment (Table 46). APG-149 recorded maximum number of roots. DR-16 recorded maximum root weight under stress treatment.

Biomass characterstics

Moisture stress significantly reduced root, leaf, stem and total biomass (Table 46). DR-16, APG-149 and APG-18 maintained higher biomass under stress compared to other genotypes.

Table 45. Effect of moisture stress on growth parameters at end of stress in cardamom

Genotype	Plant height (cm)	No. of leaves	No. of dry leaves	No. of tillers
Control				
APG-18	148.5	80.1	14.5	6.5
APG-34	132.2	40.0	8.9	7.1
APG-149	166.7	50.3	15.8	7.6
DR-3	125.4	50.2	8.7	6.3
DR-6	138.5	49.8	8.6	7.6
DR-16	175.8	42.5	14.2	8.7
Mean	147.9	52.2	10.2	7.3
Stress				
APG-18	147.1	24.9	16.3	6.2
APG-34	134.2	25.5	6.4	5.3
APG-149	149.4	27.2	19.1	4.5
DR-3	147.2	28.0	14.3	4.4
DR-6	162.5	27.3	19.9	4.9
DR-16	168.6	25.8	22.7	6.0
Mean	151.5	26.5	16.4	5.2
CD ($P<0.05$)				
Genotypes	5.5	NS	NS	NS
Treatment	NS	10.3	4.9	1.2
Interaction	NS	NS	NS	NS

Values are per clump basis

Table 46. Effect of moisture stress on root characteristics and biomass at end of stress in cardamom

Genotype	Root length (cm)	Total no. of roots	Total root wt. (g)	Total leaf wt. (g)	Total stem wt. (g)	Total biomass (g)
Control						
APG-18	29.5	41.3	321.7	318.3	1540.0	2180.0
APG-34	33.0	26.5	58.3	135.0	448.3	641.7
APG-149	38.8	29.3	146.7	331.7	1573.3	2051.7
DR-3	25.2	11.8	26.7	81.7	306.7	415.0
DR-6	26.3	21.7	53.3	228.3	750.0	1031.7
DR-16	30.4	41.2	146.7	298.3	1533.3	1958.3
Mean	30.3	28.6	122.2	232.2	1025.3	1379.7
Stress						
APG-18	25.1	17.2	25.0	126.7	620.0	771.7
APG-34	17.8	17.7	26.0	131.7	496.7	654.3
APG-149	24.2	20.3	22.5	153.3	636.7	812.5
DR-3	27.8	17.8	17.5	126.7	598.3	742.5
DR-6	25.0	14.0	13.3	116.7	405.0	535.0
DR-16	21.2	17.3	39.2	196.7	1126.7	1362.5
Mean	23.5	17.4	23.9	141.9	647.2	813.1
CD ($P<0.05$)						
Genotypes	NS	NS	63.4	NS	NS	377.4
Treatment	4.2	7.9	36.5	65.6	309.0	306.9
Interaction	NS	NS	36.6	NS	NS	NS

Values are per dump basis

Screening of germplasm in the field

Twenty-eight cardamom genotypes were evaluated for relative water content, specific leaf weight and stomatal count in the field and they recorded significant variations. Relative water content (% reduction over control) ranged from 7.26% to 33.18% with a mean of 16.33%. Specific leaf weight ranged from 4.2 to 5.8 mg/cm^2 with a mean of 4.85 mg/cm^2. Stomatal count ranged from 6.41 to 11.6 with a mean of 9.83 at 60x. The genotypes APG-275, APG-276, APG-283, APG-284, APG-289 and APG-302 recorded relatively higher relative water content under stress. The genotypes APG-276, APG-277, APG-278, APG-279, APG-284 and APG-287 recorded relatively lower stomatal counts. The genotypes APG-275, APG-278, APG-281, APG-282, APG-285 and APG-299 recorded relatively higher specific leaf weights.

Externally Funded Projects

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

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

*Centre for Water Resources Development and Management, Calicut

Tracer studies using ^{14}C-CO_3

Tracer studies were conducted to locate precursors and intermediates in curcumin synthesis. ^{14}C–labeled potassium carbonate was utilized for conducting incorporation studies of ^{14}C during very early stages of rhizome growth (up to 3 months) with regard to phenolic acids, acetate and other components. The test seedlings were placed in a perspex glass chamber (1.0 m x 0.5 m x 0.5 m) and labelled Na_2CO_3 was placed in this chamber and dilute HCl was added slowly so as to evolve CO_2. The plants were replanted in pots after exposure for 30 min. Samples were taken for standardizing methods for extraction and estimation of phenolic acids and separation through TLC to identify the individual phenolic acids.

Incorporation of phenolic acids

A gradual increase in incorporation of ^{14}C-CO_2 in phenolic acids was seen in leaves with a maximum value at 96 h after exposure (22.42 x 10^3 dpm). About 158.2% increase was seen over the initial value (7.478 x 10^3 dpm) at 48 h, which increased by 16.2% in 96 h to reach the peak value. One week after exposure, the value decreased by 51.6% and after 1 month, only 12.2% incorporation of ^{14}C-CO_2 was observed in the leaves.

V. Mega project: Value addition and post harvest processing of spices
(Project leader: T. John Zachariah)

1. Quality evaluation in spices
(T. John Zachariah, P. Heartwin Amaladhas and N. K. Leela)

Evaluation of germplasm for quality

Black pepper

The wild *Piper* collections were evaluated for oleoresin and piperine and Acc. 5411 (31.8% oleoresin and 6.2% piperine) followed by Acc. 5442 (21.6% oleoresin and 6% piperine) were identified as high quality lines.

Evaluation of chemical quality (oil, oleoresin and piperine) of black pepper obtained from *P. nigrum* grafted on *P. colubrinum* adopting techniques like saddle, approach, splice, modified splice, cleft, yemma, double and tongue grafting indicated that there was no variation in quality of black pepper berries obtained from grafts compared to ordinary rooted cuttings.

Cardamom

One hundred and fifty germplasm accessions were evaluated for essential oil and GC profile of oil (Table 47).

The husk to seed ratio varied from 22:78 to 32:68 and the oil content followed the same pattern of husk to seed ratio. A majority of the accessions were rich in α-terpinyl acetate. Among the accessions, Acc. 256 with very low 1,8 cineole and high terpinyl acetate was identified as the best quality line.

Table 47. High quality cardamom accessions

Acc.	Oil (%)	1,8-cineole (%)	α-terpinyl acetate (%)
60	8.4	25.0	28.0
75	8.3	28.7	26.4
256	7.9	15.0	25.5
273	8.3	25.0	27.3
277	7.1	24.9	30.4

Ginger

Sixty ginger accessions were evaluated for oil, oleoresin and crude fibre contents. Essential oil content ranged from 0.8% to 2.0% and oleoresin content ranged from 3.0% to 7.5%. Many accessions had relatively low fibre. Acc 121 with 1.6% oil, 7.5% oleoresin and 2.0% fibre; Gurubathani (Acc. 512) with 1.7% oil, 6.0% oleoresin and 3.9% fibre; Kozhikalan (Acc. 537) with 2.0% oil, 6.6% oleoresin and 4.1% fibre were identified as high quality accessions. Kakkakalan (Acc. 558) had 1.7% oil, 5.5% oleoresin and 3.3% fibre. The other high quality accessions are listed in the Table 48.

Table 48. High quality ginger accessions

Acc.	Oil (%)	Oleoresin (%)	Crude fibre (%)
121	1.6	7.5	2.0
260	1.5	6.3	2.8
342	1.6	6.0	1.6
512	1.7	6.0	3.9
537	2.0	6.6	4.1

Studies on effect of maturity on starch content in three cultivars/varieties of ginger namely, Maran, Varada and Himachal, indicated that there was steady increase in starch content as maturity increased. However, high accumulation of starch took place from 180 days onwards which coincided with maximum accumulation of oil and oleoresin.

Cassia

Nineteen cassia accessions were evaluated for oil and oleoresin. Oil content varied from

1% to 7% and oleoresin content varied from 5% to 11%. A-2 (oil 6.0%, oleoresin 11.2%), D-3 (oil 5%, oleoresin 11%) and C-1 (oil 6.0%, oleoresin 8.8%) were identified as high quality accessions.

Externally Funded Projects

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

(*B. Chempakam and T. John Zachariah*)

Drying characteristics of black pepper

Three black pepper varieties/cultivars (P-24, Panniyur-1 and Karimunda) were dried in agricultural waste-fired batch type CPCRI small holder's dryer. Drying was accomplished after 9 h of exposure, with a dry recovery of 36.4%, 32.3% and 33.3% for P-24, Panniyur-1 and Karimunda, respectively. Sun drying which took 4 to 5 days to achieve complete drying; also exhibited the same pattern of dry recovery. The quality of berries was analysed for moisture, volatile oil, oleoresin and piperine contents (Table 49).

Preparation of white pepper

Seven promising bacterial isolates (Is. nos. 2, 9, 19, 22, 27, 30 and 55) were selected to study the suitability of individual bacteria as pure cultures on preparation of white pepper. The black pepper berries were kept in boiling water for 10 min and were treated with the selected bacterial strains. The heating process facilitated microbial degradation of the pericarp of the berries and the incubation period was reduced to 3 days from 5 days. But the characteristic colour of the white pepper could not be obtained.

The effect of preparing white pepper from black pepper berries on microbial flora, adopting the pit method was studied. Two samples of black pepper berries were selected and one sample was kept in boiling water for 10 min and the other was not given any treatment. Both the samples were buried in 60 cm deep soil for 15 days. The samples were taken out and examined for microbial counts at regular intervals (Table 50). The microbial population did not exhibit much variation between heated and non-heated samples.

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*)

Documentation of indigenous technologies

Various black pepper farms were visited and indigenous systems available for despiking were documented.

Table 49. Effect of drying methods on quality of black pepper

Variety/Cultivar	Moisture (%)	Volatile oil (%)	Oleoresin (%)	Piperine (%)
Hot air dried black pepper				
Panniyur-1	9.8	3.4	8.9	2.9
P-24	9.3	3.8	10.2	3.2
Karimunda	9.0	3.4	9.4	3.1
Sun dried black pepper				
Panniyur-1	10.1	3.2	9.7	2.7
P-24	9.5	4.4	12.3	2.9
Karimunda	9.8	3.6	10.4	3.1

Table 50. Microbial flora associated during preparation of white pepper

Duration	Heated		Not heated	
	Bacteria (log cfu)	Fungi (log cfu)	Bacteria (log cfu)	Fungi (log cfu)
Control (0 day)	7.29 b	5.01 c	8.43 b	5.98 c
5th day	9.18 a	6.98 a	9.22 a	6.93 ab
10th day	9.28 a	6.80 b	9.31 a	6.96 ab
15th day	9.24 a	6.93 ab	9.14 a	6.90 ab

Data with same letter designation are not significant according to DMRT at $P<0.05$
cfu = Colony forming units

Packaging systems

A zero energy chamber (a double walled brick structure filled with sand between the walls, frequently moistened) was fabricated for storing fresh ginger rhizomes (Fig. 18). Storing freshly harvested ginger (in wooden box lined with polyethylene with 1% ventilation) in the chamber kept the rhizomes healthy for 4 months (Fig. 19). The reduction in weight was 23% compared to 50% in open and 40% in ordinary polyethylene cover.

Storage of dry ginger and turmeric

Storage of ginger with dry leaves of *Azadirachta indica*, *Glycosmis cochinchinenesis* and *Clerodendron infortunatum* prevented attack of storage pests. Long term packing in vacuum also helped storage of dry turmeric without pest damage.

Fig. 18. Zero energy chamber for storing fresh ginger

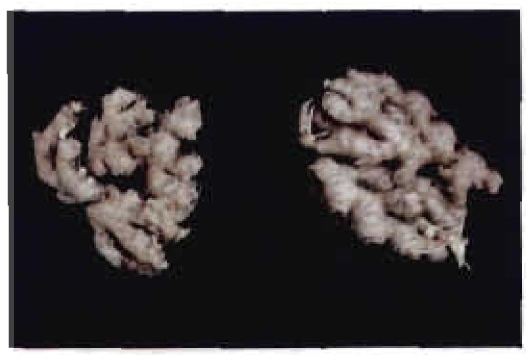

Fig. 19. Ginger rhizomes stored in open conditions (left) and in zero energy chamber (right)

Final Report

1. ICAR: Characterization of nutmeg germplasm for quality

(*B. Krishnamoorthy and T. John Zachariah*)

Objectives

- Characterization of nutmeg germplasm for quality.
- Identification of high quality nutmeg accessions from the germplasm.

Major quality constituents

Essential oil of nutmeg and mace of 95 accessions was estimated by Cleavenger method. The essential oil ranged from 3.9% (A9-116) to 16.5% (A9-18) in nutmeg and from 6.0% (A9-107) to 26.1% (A9-18) in mace. Accessions with high nutmeg oil (>12%) were A9-36, A11-46, A4-50, A11-49, A11-15, A9-49 and A9-18 and those with high mace oil (greater than 17%) were A4-11, A11-38, A11-49, A9-49 and A9-18.

Constituents of essential oil

The major components in nutmeg and mace oils are α-pinene, sabinene, safrole, myristicin and elemicin. α-pinene has terpentine-like odour, sabinene sweet odour, and myristicin warm balsamic odour. Sabinene enhances the sweetness of the product to which it is added. Myristicin, elemicin and safrole are the hallucinogenic principles of nutmeg. Accessions with high sabinene and low myristicin and elemicin are desirable for confectionery. Myristicin has now been identified as an active principle that could be utilized in prevention of cancer. Hence, accessions which are rich in myristicin have application in pharmaceutical industry. The essential oils of nutmeg and mace from 65 accessions were analysed by Gas Liquid Chromatography and the percentages of myristicin, elemicin, safrole, α-pinene, and sabinene fractions in nutmeg and mace oils were calculated (Table 51).

In nutmeg oil, myristicin content ranged from 0.3% (A9-13) to 45.6% (A11-21), elemicin from 0.4 % (A9-107) to 30.9 % (A9-85) and safrole from 0.1% (A9-4) to 22.1% (A9-13). α-pinene in nutmeg oil ranged from 1.6% (A9-4-11) to 23.5% (A9-102) and that of sabinene from 6.3% (A9-1) to 50.2% (A9-44).

In mace oil, myristicin content ranged from 0.2% (A9-13) to 36.6 % (A4-117), elemicin from 0.4% (A9-74) to 30.2% (A4-12) and safrole from 0.2% (A4-5) to 21.8% (A9-13). α-pinene in mace oil varied from 1.5% (A4-12) to 21.1% (A9-3) and sabinene from 5.9% (A4-12) to 43.4% (A11-13). A4-17 had high myristicin in both nutmeg and mace oils.

Accessions A9-71 and A9-95 had low myristicin, elemicin and safrole coupled with high sabinene in both nutmeg and mace oils and these two accessions are suitable for preparing confectionery. A9-18, A9-4 and its progenies had high myristicin content in both nutmeg and mace oils and have application in pharmaceutical industry.

Table 51. Classification of nutmeg accessions based on essential oil constituent

Accs.	Category	Remarks
A4-17, A4-20, A9-4-12, A9-4-13 and A9-4-15	High myristicin and elemicin in nutmeg oil	-
A4-17, A9-4-1, A9-4-3, A9-4-8 and A9-4-11	High myristicin and elemicin in mace oil	-
A4-17	High myristicin in nutmeg and mace oils	Suitable for pharmaceuticals
A4-22, A9-69, A9-71, A9-95 and A9-102	Low myristicin and elemicin in nutmeg oil	-
A9-1, A9-44, A9-71 and A9-95	Low myristicin and elemicin in mace oil	-
A9-71 and A9-95	Low myristicin, elemicin and safrole and high sabinene in nutmeg and mace oils	Suitable for confectionery

Butter and oleoresin

The most prominent fatty acid in nutmeg butter is myristic acid. Butter content ranged from 21.5% (A9-107) to 44.0% (A11-12). The oleoresin content in nutmeg (obtained with acetone) ranged from 1.4% to 5.3%. However, when ethanol was used, the yield of oleoresin increased from 6.7% (A9-1) to 23.0% (A9-116). Oleoresin content in mace (with acetone) ranged from 13.8% (A9-1) to 32.2% (A4-22).

Leaf essential oil and its constituents

Essential oil in leaf ranged from 0.98% (A9-26) to 2.50% (A11-70) (v/w), myristicin ranged from 0.3% to 11.9% and that of elemicin ranged from 0.30% to 7.98%. There was no correlation between essential oil profile of leaf and sex of the plant.

Amino acid

Leaf amino acids from male, female and bisexual plants were analysed by HPLC to check whether the profile could be used for identification of the sex of the plant. However, there was no difference in the amino acid profile with reference to the sex of the plant. Phenylalanine was the predominant amino acid in most of the accessions and ranged from 39.86 to 442.85 µg/mg. Alanine was also a prominent amino acid in these accessions and ranged from 25.24 to 80.93 µg/mg. Leaf amino acids from related taxa of *Myristica* were also analysed and phenylalanine was the prominenet amino acid in most of the species with a range of 6.38 to 315.56 µg/mg.

Free amino acid

No significant difference was observed in the total free amino acid content in leaf samples of various accessions. Among the different species of *Myristica*, *M. fatua* var. *magnifica* had the lowest content (127.65 µg/100 mg) of total free amino acids while the highest amino acid content was observed in *M. prainii* (315.21 µg/100 mg).

Non-volatile ether extract

The non-volatile ether extract of nutmeg and mace was estimated by AOAC method and was found to be 33.77% and 18.65%, respectively.

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 (55,000), turmeric seed rhizomes (1.5 t), ginger seed rhizomes (3 t), cardamom seedlings (28,000), cardamom seed capsules (50 kg) and nutmeg grafts (11,100) were produced and distributed to farmers and other agencies (Fig. 20).

Fig. 20. Production of nucleus planting materials of high yielding turmeric 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)

Thirty-nine black pepper gardens in Kodagu District (Karnataka) and 22 black pepper gardens in Wyanad district (Kerala) were surveyed for the incidence of stunt disease and the insect fauna associated with it. The incidence of the disease ranged from 0% to 100% in the gardens surveyed and was higher in Wyanad District than Kodagu District. Mosaic, small leaves and reduced internodal length leading to stunting of vines were the typical symptoms observed in a majority of the gardens in Wyanad while mottling along the veins and mosaic were the symptoms observed in Kodagu. Leaf gall thrips, coconut scale, mussel scale, root mealybug and citrus aphid were the insect fauna associated with diseased vines. Forty virus isolates were collected from Karnataka, Kerala and Tamil Nadu and are being maintained under insect proof conditions for further studies.

Association of a badn avirus serologically related to banana streak virus (BSV) with diseased black pepper vines showing yellow mottle symptoms from Kozhikode and Wyanad districts was established for the first time based on transmission, electron microscopy and serology using Direct Antigen Coated-ELISA (DAC-ELISA). The virus was transmitted from diseased to healthy black pepper plants using the striped mealybug, *Ferrisia virgata* with 24 h acquisition access and inoculation access periods. The inoculated plants showed typical symptoms of the disease within 5 weeks after inoculation. Electron microscopy of partially purified preparations from naturally infected black pepper leaves showed the presence of bacilliform shaped particles. In DAC-ELISA, diseased samples showed positive reaction with banana streak and sugarcane bacilliform badnaviruses (Table 52). The exact taxonomic status of the causal virus is yet to be determined.

The cucumber mosaic virus (CMV) infecting black pepper was easily sap transmitted onto several species of tobacco and other solanaceous and cucurbitaceous hosts. A protocol was standardized for the purification of CMV from tobacco. The purified preparation showed the presence of typical isometric particles under electron microscope (Fig. 21). Polyclonal antiserum against CMV is being produced by injecting purified preparations into New Zealand white rabbits.

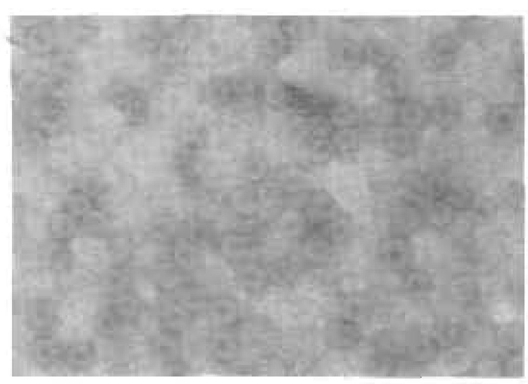

Fig. 21. Electron micrograph of purified preparations of cucumber mosaic virus

Table 52. Detection of badnavirus in field infected black pepper plants in DAC-ELISA*

Locality	A_{405} values against antisera to	
	BSV	ScBV
Kozhikode		
Sample 1	0.17	0.10
Sample 2	0.19	0.11
Sample 3	0.33	0.09
Sample 4	0.56	0.12
Sample 5	0.11	0.11
Sample 6	0.22	0.19
Sample 7	0.19	0.11
Sample 8	0.20	0.13
Wyanad		
Sample 1	0.10	0.13
Sample 2	0.48	0.19
Sample 3	0.44	0.28
Sample 4	0.12	0.16
Sample 5	0.10	0.09
Sample 6	0.22	0.19
Sample 7	0.26	0.21
Sample 8	0.31	0.19
Healthy black pepper	0.04	0.07

*Average of three replications, 1 h after substrate addition; BSV = Banana streak virus; ScBV = Sugarcane bacilliform virus

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

(M. Anandaraj, M. N Venugopal, K. S. Krishnamurthy, V. Srinivasan, D. Prasath, K. Kandiannan and R. Ramakrishnan Nair)

Two new field trials was laid in a farmer's plot at Appangala (Kodagu District) to study the effect of fungicides, naphtheline acetic acid (NAA), gibberellic acid (GA), fluorescent *Pseudomonas*, micronutrients and potash on the field incidence of anthracnose, spike shedding and yield of black pepper. Monthly observations on the ratio of male and female flowers in Panniyur-1 vines at high altitudes were recorded and a low percentage of bisexual flowers was observed during August (3.9%) (Table 53). This could be one of the reasons for sparse setting and spike shedding in Panniyur-1, particularly at high altitudes. The corresponding bisexual status of flowers in Panniyur-1 located in moderate rainfall and low altitude areas was 68%. The incidence of spike shedding was less in all fungicide treated plots compared to untreated control plots (Table 54). The experiment with growth hormones and micro nutrients indicated that the yield was higher in treated plots and the number of spikes required for 1 kg fresh weight of spikes was lesser indicating that fruit set was higher in treated plants than control (Table 55).

Table 53. Production of bisexual flowers in black pepper *var.* Panniyur-1

Month	Bisexual flowers (%)
May	88.0
June	90.5
July	31.5
August	3.9
September	22.7
October	60.8
November	72.5

The reaction of black pepper cultivars/varieties to natural infection of anthracnose was recorded in the area with known history of spike shedding and Panniyur-1 was the most susceptible and Panniyur-5, Subhakara, Panchami, Balankotta and Kottanadan were tolerant to natural infection of anthracnose.

3. Investigation on vein clearing virus of small cardamom

(M. N. Venugopal and A. Ishwara Bhat)

Electron microscope studies

Leaf and pseudostem samples were collected from inoculants of different stages

Table 54. Effect of fungicides and *Pseudomonas fluorescens* on anthracnose of black pepper

Treatment	Disease index (%)	Fresh yield (kg/plant)	Spikes/kg (fresh wt.)
Bordeaux mixture (1%)	31	1.89	144.0
Bordeaux mixture (0.5%)	44	1.38	177.5
Bordeaux mixture + Hexaconozole	42	1.81	158.3
Hexaconozole	40	1.57	156.3
Hexaconozole + Bordeaux mixture (1%)	45	1.28	158.8
Carbendazim (0.2%)	28	2.14	151.0
Mancozeb (0.25%) + Carbendazim (0.2%)	36	1.52	158.8
Carbendazim (0.2%) + Mancozeb (0.25%)	31	1.79	146.8
Mancozeb (0.25%)	34	1.58	168.3
Zineber (0.25%)	37	1.66	164.8
Pseudomonas fluorescens	42	1.38	171.0
Potassium phosphonate	35	1.61	151.5
Control	54	1.34	149.8
CD ($P<0.05$)	-	0.24	13.1

Table 55. Effect of growth regulators and micronutrients on yield of black pepper

Treatment	Fresh yield/ plant (kg)	No of spikes/ kg fresh wt.
NAA	4.205	146.50
GA	3.456	162.00
K + Zn + B + NAA	3.963	156.80
K + Zn + B + GA	2.997	138.80
Control	2.400	178.80
CD ($P<0.05$)	0.475	0.13

NAA = Naphtheline acetic acid; GA = Gibberellic acid; K = Potash; Zn = Zinc; B = Boron

and leaf dip preparations were examined for presence of virus. However, the samples did not reveal the association of any virus.

Serology through DAC-ELISA

Serological relationship of *kokke kandu* virus was studied by using antiserum prepared against PVY (potato virus Y), HMV (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.

Screening germplasm and disease escapes

Final screening of disease escapes with viruliferous aphids carrying *kokke kandu* virus (Sirsi isolate) was undertaken. Twelve disease escapes were short-listed for next phase of field testing in hot spots. Twelve short-listed hybrids, 11 mosaic resistant plants and 12 disease escapes were multiplied for field screening in hot spots.

4. Studies on bacterial wilt of ginger

(A. Kumar and R. Suseela Bhai)

Rhizome solarization

Effect of heat on survival of Ralstonia solanacearum

The thermal death point (TDP) and thermal death time (TDT) of *R. solanacearum* was determined using thermal cycler. The TDP for *R. solanacearum* was 45.8°C at 30 min

(TDT) of exposure (Tables 56 and 57). However, the bacterium was completely killed even at 10 min exposure at 50.8°C.

Optimization of time and duration of rhizome solarization

In order to optimize the time of day for solarizing rhizomes to raise the rhizome temperature, trials were conducted during three different times of a day (Table 58). The trials indicated that solarization can be done at any time in a day; however, depending upon the time, the duration should be adjusted so as to get optimum temperature for inactivation of viable cells of *R. solanacearum* in the rhizomes. Adequate precaution should be taken to stop the treatment, 30 min after attaining the rhizome temperature of 48°C in any time of a day.

Table 56. Optimization of thermal death point and thermal death time for *Ralstonia solanacearum*

Temperature	Duration of exposure (min)					
(°C)	5	10	15	20	25	30
40.0	+	+	+	+	+	+
40.2	+	+	+	+	+	+
41.0	+	+	+	+	+	+
42.5	+	+	+	+	+	+
44.3	+	+	+	+	+	+
46.4	+	+	-	-	-	-
48.6	+	+	-	-	-	-
50.8	-	-	-	-	-	-
52.8	-	-	-	-	-	-
54.5	-	-	-	-	-	-
55.8	-	-	-	-	-	-
56.6	-	-	-	-	-	-

+ Growth of *R. solanacearum*; - No growth

Table 57. Optimization of thermal death point and thermal death time for *Ralstonia solanacearum*

Temperature	Duration of exposure (min)
(°C)	30
44.5	+
44.6	+
44.7	+
44.8	+
44.9	+
45.0	+
45.1	+
45.2	±
45.3	±
45.4	±
45.5	±
45.6	±
45.7	±
45.8	±
45.9	-
46.0	-
46.1	-
46.2	-
46.3	-
46.4	-
46.5	-
28.0	+

+ Growth of *R. solanacearum*; ± Slow growth ; - No growth

Table 58. Effect of time of solarization on rhizome temperature of ginger

Duration of solarization	Rhizome temperature (°C)		
(min)	Time of solarization		
	10:00–11:00 hrs	13:00–14:00 hrs	15:00–16:00 hrs
0	29.00	29.00	30.00
15	35.60	40.90	39.00
30	42.00	48.70	44.50
45	45.00	52.80	45.00
60	48.30	51.10	44.50
Rate of heat build up (°C per min)	0.32	0.37	0.24

Effect of rhizome solarization on viability of rhizomes

One month of storage soon after rhizome solarization did not affect germination of ginger rhizomes. However, long exposure (beyond 2 h) affected the firmness of the rhizomes (Table 59). This trial confirmed that a temperature above 50°C is injurious to the sprouts.

Table 59. Storability of solarized (heat-treated) ginger rhizomes

Duration of solarization (min)	Temp. at end of exposure (°C)	Viable sprouts (%)
0	28.7	97 (80.9)[a]
15	41.0	99 (83.6)[a]
30	43.3	95 (78.1)[a]
45	47.0	98 (82.0)[a]
60	53.7	54 (47.2)[b]
75	52.7	4 (10.3)[c]
90	54.3	3 (8.4)[c]

Data with same letter designation are not significant according to DMRT at $P<0.05$; Figures in parenthesis are arc sin transformed values

Effect of rhizome solarization on heat retention in rhizomes

The effect of rhizome solarization on heat build up in rhizomes and consequent heat retention was worked out to study rhizome viability. The heat was retained in the rhizomes for 30 min (Table 60).

Integrated management of bacterial wilt

Effect of rhizome solarization on bacterial wilt

A trial with six heat treatments through rhizome solarization was conducted at Peruvannamuzhi for the management of the disease. Rhizomes exposed to sunlight for 60 min (48.3°C) and 75 min (45.3°C) recorded 100% healthy plantlets as compared to 13.3%–86.7% wilt incidence under unexposed and exposed (29.0–43.2°C) treatments. A rhizome yield ranging from 165.8 to 234.2 g/plant was obtained in these treatments (Table 61).

Screening of ginger accessions

Over 250 ginger accessions from the germplasm were screened for bacterial wilt tolerance using soil inoculation method by four different (decimal dilutions) concentrations of bacterial inoculum. All the collections wilted after 2 months of inoculation. However, few accessions (Accs. 366, 351, 375, 402, 484, 493, 514, 524, 557 and 589) regenerated and such rhizomes were maintained for further screening.

Characterization and detection of* Ralstonia solanacearum *in soil

Characterization of Ralstonia solanacearum

Six new collections of *R. solanacearum* were added to the repository. Rep-PCR analysis

Table 60. Effect of rhizome solarization on rhizome temperature and heat retention in ginger

*Initial rhizome temp. (°C)	Temp. after 30 min (°C)	Reduction in heat after 30 min	Temp. after 2 h (°C)	Reduction in heat after 2 h
29.0	29.0	0	29.0	0
41.3	35.6	5.7 (0.19)	33.8	7.5 (0.063)
43.2	36.2	7.0 (0.23)	32.9	10.3 (0.086)
48.3	36.9	11.4 (0.38)	32.1	16.2 (0.140)
45.3	37.8	7.5 (0.25)	33.5	11.8 (0.098)

Figures in parenthesis are rate of heat reduction per min

* Temperature recorded after solarization for 0, 15, 30, 45 & 60 min

Table 61. Effect of rhizome solarization (heat treatment) on bacterial wilt incidence and yield of ginger in greenhouse

Treatment	Temperature at end of exposure (°C)	Bacterial wilt incidence after 4 months (%)	Yield (g/plant)
Untreated	29.0	86.7 (72.4)[b]	0.0[b]
30 min	41.3	31.4 (29.9)[a]	193.3[a]
45 min	43.2	13.3 (17.6)[a]	146.7[a]
60 min	48.3	0.0 (6.8)[a]	234.2[a]
75 min	45.3	0.0 (6.8)[a]	209.2[a]
Healthy rhizomes	29.0	0.0 (6.8)[a]	165.8[a]

Data with same letter designation are not significant according to DMRT at $P<0.05$; Figures in parenthesis are arc sin transformed values

grouped the isolates (28) into four major haplotypes with a similarity coefficient of 0.70; two clusters caused bacterial wilt of ginger. ITS-PCR (using primer ITS ALL F) and RAPD (using primer OPA-2) analysis further confirmed the narrow genetic base of bacterial wilt pathogen, which indicated that the pathogen is primarily rhizome-borne and rhizome movement across the country is responsible for the spread of the disease.

Molecular approaches for pathogen detection in soil

Isolation of DNA from soil and plant debris

A protocol was refined for isolation of DNA from soil. The protocol yielded PCR amplifiable DNA with the A260/280 ratio ranging from 1.51 to 2.09 and A260/230 ratio ranging from 0.53 to 2.14. The DNA yield ranged from 0.095 to 1.840 μg per g of soil. The soil (bacterial) DNA isolated was restriction digestible.

PCR based detection of Ralstonia solanacearum

R. solanacearum was detected in soil at a concentration of 10^4 cells per g soil using PCR. *R. solanacearum* could be selectively detected in the presence of high population of fluorescent pseudomonas IISR-51 and IISR-6 in a co-cultured soil.

5. Studies on fungal and viral diseases of ginger

(*R. Suseela Bhai, A. Ishwara Bhat and Santhosh J. Eapen*)

Identification of pathogens

Macrophomina sp. was identified as the causative organism of dry rot disease and *Fusarium oxysporum* as the causal organism of eye rot disease of ginger, which occurrs during the post harvest period (Fig. 22). *Colletotrichum* sp., *Phoma* sp. and *Pestalotiopsis* sp. were isolated from leaf spot specimens collected from Peruvannamuzhi, Wyanad and Kannur, respectively, and their pathogenicity was proved.

Fig. 22. Dry rot of ginger rhizomes

Etiology

Studies in relation to disease development using integrated disease management strategies to understand the etiology of dry rot and eye rot diseases indicated that planting unhealthy or diseased rhizomes leads to disease even after seed treatment. Treatment of diseased rhizomes with steam was not advisable, as it killed the meristamatic buds.

Screening of fungicides

Seven fungicides at four concentrations were tesed against various pathogens (*Fusarium oxysporum*, *Pythium myriotylum*, *Phoma* sp., *Pestalotiopsis* sp. and *Macrophomina* sp.) and the ED_{50} values were determined.

Carbendazim and SAAF [a combination of Mancozeb (63%) + Carbendazim (12%)] were inhibitory even at 50 ppm of the product when compared to Mancozeb, Ridomil, Bordeaux mixture or copper oxychloride, which had no effect even at 500 ppm concentration (Table 62).

Screening of ginger accessions

Two hundred and fifty germplasm accessions were screened against *Pythium* sp. causing soft rot disease. Thirty-three accessions that escaped the infection in the preliminary screening were subjected for secondary screening and five accessions namely, Accs. 6, 17, 130, 155 and 208 were relatively tolerant. Among the 650 germplasm accessions of ginger evaluated under natural conditions, 281 accessions were infected by chlorotic leaf streak virus.

Externally Funded Projects

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

(*M. Anandaraj and Y. R. Sarma*)

Characterization of *Phytophthora*

Five hundred and fourteen isolates of *Phytophthora* from 35 crops are being maintained in the National Repository of *Phytophthora*. One hundred and seventy-two isolates of *Phytophthora* from black pepper were characterized morphologically. Among them, 161 isolates belonged to *P. capsici*, 4 to *P. palmivora*, 3 to *P. parasitica*, 2 to *P. meadii* and 2 were atypical isolates. *Phytophthora* was isolated from new hosts like tapioca, vanilla, bauhinia, nutmeg and *Piper chaba*. *P. capsici* was able to grow at temperatures varying from 10°C to 35°C. The white cottony type of isolates did not grow at 10°C and four white cottony types

Table 62. Evaluation of fungicides against pathogens of ginger

Fungicide	ED_{50} (ppm)				
	Fusarium oxysporum	*Macrophomina phaseolina*	*Phoma* sp.	*Pestalotipsis* sp.	*Pythium myriotylum*
Metalaxyl	1250	-	609	1172	100
Mancozeb	1070	-	667	872	-
Copper oxychloride	-	-	1125	1072	250
Carbendazim	50	50	50	50	726
Bordeaux mixture	-	100	250	560	192
Mancozeb + Carbendazim	50	50	50	50	926

were not able to grow at 35°C. Isolates with chrysanthemum and stellate pattern could grow at 10°C and 35°C. Maximum sporulation was at 28°C, followed by 20°C. Sporulation was very less at 10°C and 35°C. *Phytophthora* could be successfully stored in sterile distilled water and retrieved up to 7 months without loosing its viability.

Fifty-two isolates of *Phytophthora* obtained from betel vine were characterized morphologically and both *P. capsici* and *P. parasitica* were determined to be involved in foot rot of betel vine. Isozyme studies on betel vine isolates of *Phytophthora* confirmed the presence of two distinct species namely, *P. capsici* and *P. parasitica* as suggested by morphological characterization. Variability among isolates of *P. capsici* obtained from betel vine was negligible. However, among the *P. parasitica* isolates from betel vine, the West Bengal isolates were very distinct from the isolates collected from other regions, confirming their distinct identity based on morphological characters. The *P. parasitica* isolate, 98-119, behaved very distinctly from all the other isolates and could be considered as an out-group.

UPGMA cluster analysis of isozyme data of black pepper *Phytophthora*, grouped the isolates into a major cluster consisting of typical *P. capsici* isolates and three other clusters consisting of either off-types or *P. capsici* isolates that differed in isozyme patterns from the majority of the typical isolates. The *P. capsici* isolates which clustered together were grouped into two major sub-groups. The first sub-group generally consisted of highly virulent isolates which had sporangia with a L/B ratio of 2 or >2. The second sub-group consisted of isolates with L/B ratios of their sporangia, less than or equal to 2 and composed of less, moderate and highly virulent types. This sub-group consisted of three clusters of isolates with a general L/B ratio of values less than or equal to 2 and differed from each other based on their virulence. The sterile isolates were 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, and had a modified chrysanthemum-like colony character. The isolates identified morphologically as off-types, and lacking the typical characters of *P. capsici*, grouped differently from the major cluster of *P. capsici* and among them, *P. palmivora* and *P. parasitica* formed separate groups. The black pepper isolates with modified chrysanthemum-type colony morphology formed a unique sub-group among *P. capsici* isolates based on isozyme studies. It is postulated from this study that this group could be considered as similar to the *Cap A* group described by Mchau and Coffey (1995) as the isolates in this group have sporangia with a L/B ratio of 1.5 which is closer to the dimensions suggested by these authors for the *Cap A* isolates. Further, the rest of the *P. capsici* isolates with a L/B ratio of around 2 or >2 could be considered as *Cap B* isolates.

Protocols were standardized for RAPD analysis of *Phytophthora* of black pepper and cardamom. Out of 20 random primers tested, primer OPA-09 amplified a DNA (872–1353 bp) in foot rot tolerant cultivars of black pepper that were screened for RAPD analysis.

Induction of pathogenesis related proteins (PR-proteins) and defence related enzymes were observed in infected tissues of

Phytophthora tolerant black pepper lines. Western blot analysis confirmed the occurrence of chitinase and β-1,3 glucanase, which exhibited antifungal activity against *P. capsici* in *in vitro* studies. Accumulation of callose was noticed in leaves of P-24, a tolerant line, on infection.

Evaluation of antagonists

Over 500 antagonists of *Phytophthora* namely, *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., *Bacillus* spp. and *Pseudomonas fluorescens* were isolated from rhizospheres of black pepper, ginger, citrus, betel vine and cardamom. At present the Repository of Biocontrol Agents contains 585 fungal and 832 bacterial antagonists. Isolates of *Trichoderma* spp. (221) and bacteria (196) were screened for their antagonism to *P. capsici* by adopting dual plate technique. Among *Trichoderma* spp., the percentage of inhibition of *P. capsici* varied from 0% to 84% and 34 isolates of bacteria were able to inhibit *P. capsici* recording more than 50% inhibition. In glasshouse experiments involving 79 isolates of *Trichoderma* short-listed based on *in vitro* screening, 28 isolates were highly efficient in growth promotion of black pepper. Eight isolates were able to suppress *P. capsici* in soil.

Volatiles of *Trichoderma* spp. adversely affected the virulence of *P. capsici* in *in vitro* experiments. Volatile affected *P. capsici* isolates produced lesions of smaller diameter than non-volatile affected isolates and the reduction in virulence ranged from 0% to 100%. The loss of virulence of *P. capsici* was dependent on type of *Trichoderma* isolate used and also the duration of exposure. Out of 20 *T. harzianum* isolates studied, 6 caused more than 50% reduction in virulence of *P. capsici*.

T. harzianum P-26, *T. virens* P-12, *T. aureoviride* 25 and *T. virens* 17 were evaluated to determine antagonist dose and disease incidence relationship. When no antagonist was present (0 level) the disease incidence was 90%. An antagonist dose is considered effective when there is only 50% incidence of disease or below. Accordingly, in the test using *T. harzianum* P-26, there was reduction in disease incidence at an antagonist dose as low as 10^3 cfu/g of soil and the incidence was almost same even at higher doses. Isolate *T. virens* P-12 required 10^8cfu/g of soil for effective suppression of the pathogen. Isolate *T. aureoviride* 25 also required 10^7 cfu/g of soil to reduce the disease incidence. *T. virens* 17 reduced the disease incidence at an antagonist dose of 10^4 cfu/g of soil. Application of a mixture of *Trichoderma* spp. enhanced growth of black pepper and suppressed foot rot.

A technique for isolation of protoplasts from *Trichoderma* spp. was standardized and protoplast regenerated colonies that were able to grow at higher concentrations of fungicides were isolated. *T. virens* P-12 protoplasts tolerant to 300 ppm of copper oxychloride, *T. auroeviride* protoplasts tolerant to 800 ppm of metalaxyl MZ and *T. harzianum* P-26 protoplasts tolerant to 500 ppm of metalaxyl MZ were obtained.

Multiplication of antagonists

On-farm multiplication of *T. harzianum* using organic matter was studied and population increase was observed at all the different proportions of initial inoculum levels evaluated (1:25, 1:50, 1:100, 1:200) which was similar at different locations. *T. harzianum* and organic material in the ratio of 1:100 was found ideal. Soil amended with organic materials like neem cake, coir pith, coir pith compost, farm yard manure and *Gliricidia* leaves showed better growth and survival of antagonists than in soil alone.

T. harzianum and *T. virens* were produced by liquid fermentation in 100 l capacity fermenter by utilizing potato sucrose broth and molasses. The population of *T. harzianum* after 72 h in 75 l potato sucrose broth attained 20 x 10^5 cfu/ml and the same for *T. virens* was 9 x 10^5 cfu/ml. In molasses medium, the population of *T. harzianum* and *T. virens* attained was 37.6 x 10^{10} and 7 x 10^{10} cfu/ml, respectively, after 72 h. Propagules of *T. harzianum* proliferated well in coir pith, coir pith compost and lignite under sterile and non-sterile conditions. The population was high in coir pith and coir pith compost followed by lignite. Furthermore, the population was high in sterile conditions of coir pith and coir pith compost than non-sterile conditions. But there was no difference in the population in sterile and non-sterile lignite.

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

(Project leader: M. Anandaraj)

1. Screening black pepper germplasm for reaction to diseases

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

Six hybrids, which showed tolerant reaction to *Phytophthora capsici*, were screened against various isolates of *P. capsici* using stem and leaf inoculation techniques. The hybrids exhibited varying reactions with isolates of *P. capsici* under uniform conditions of inoculation.

Seventy hybrids were screened against *P. capsici* using stem inoculation method in the greenhouse. Seven hybrids (HP-9, HP-117, HP-477, HP-528, HP-561, HP-599 and HP-1660) showed tolerant reaction with a disease index between 3.4–4.0.

Twenty-five cultivars were subjected to secondary screening and 3 cultivars (C-888, C-1204, and C-1199) showed tolerant reaction.

Sixteen Kottanadan selections were screened to confirm their tolerance and nine accessions (Accs. 2420, 2425, 2426, 2428, 2432, 2433, 2466, 2535 and 2575) were tolerant.

2. Screening black pepper germplasm for reaction to nematodes

(*K. V. Ramana, Santhosh J. Eapen and K. V. Saji*)

Eighty-six black pepper hybrids were multiplied for screening and carrot cultures of *Radopholus similis* and *Meloidogynae incognita* were maintained. Thirty-six germplasm accessions were screened against *M. incognita* and 8 accessions (hybrids-3, wild-2, cultivars-3) showed resistant reaction. A new trial was laid out in the greenhouse to confirm the resistance of 21 black pepper germplasm accessions.

3. Screening black pepper germplasm for reaction to insect pests

(*K. M. Abdulla Koya, S. Devasahayam and K. V. Saji*)

The black pepper germplasm maintained at Peruvannamuzhi, and somaclones at Chelavoor, were subjected to screening against *pollu* beetle (*Longitarsus nigripennis*) to locate sources of resistance against the pest. Out of 162 cultivars screened, three were free of infestation (Accs. 461, 1118 and 4109). In the rest of the cultivars, the infestation ranged from 0.8% to 38.5% on the berries. None of the 26 hybrids screened were resistant to the pest and the infestation on berries ranged from 3.0% to 88.3%. All the three somaclones were highly susceptible to the pest. Laterals of 7 accessions and 4 hybrids that were found comparatively tolerant to *pollu* beetle earlier were produced for subjecting to screening under field conditions.

4. Mechanisms of resistance to pests and pathogens in spice crops

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

RAPD profiling of *pollu* beetle (*Longitarsus nigripennis*) resistant lines (Accs. 816, 841, 1114 and 2070) along with two susceptible controls (P-24 and Panniyur-1) was done using 30 primers. Twenty-eight of these primers showed amplification and the number of bands ranged from 8 to 13. Five primers showed specific bands in one of the collections. One of the primer, OPAA-08 showed specific band in resistant lines only and OPAB-02 in susceptible lines. The data showed that the resistant lines formed a separate cluster from the susceptible lines (Fig. 23). The primer sequences of OPAA-08 were matched with already reported sequences of insect resistant genes by BLAST search. There were five matches out of which three are patented insect resistant genes and two *Bt* sequences. The leads obtained in this study could be used to develop SCAR markers for screening for *pollu* resistance at seedling stage itself.

Externally Funded Project

1. DBT: Immunological approaches for pathogen detection and use of defence proteins in disease management in plantation crops: ginger and cardamom

(A. Kumar, B. Sasikumar, M. N. Venugopal and Y. R. Sarma)

Biovar specific proteins from **Ralstonia solanacearum**

High molecular weight membrane protein (42.3 kDa) specific for biovar III of *Ralstonia solanacearum*, the bacterial wilt pathogen of ginger was purified by native gel electrophoresis. Polyclonal antibodies were devel-

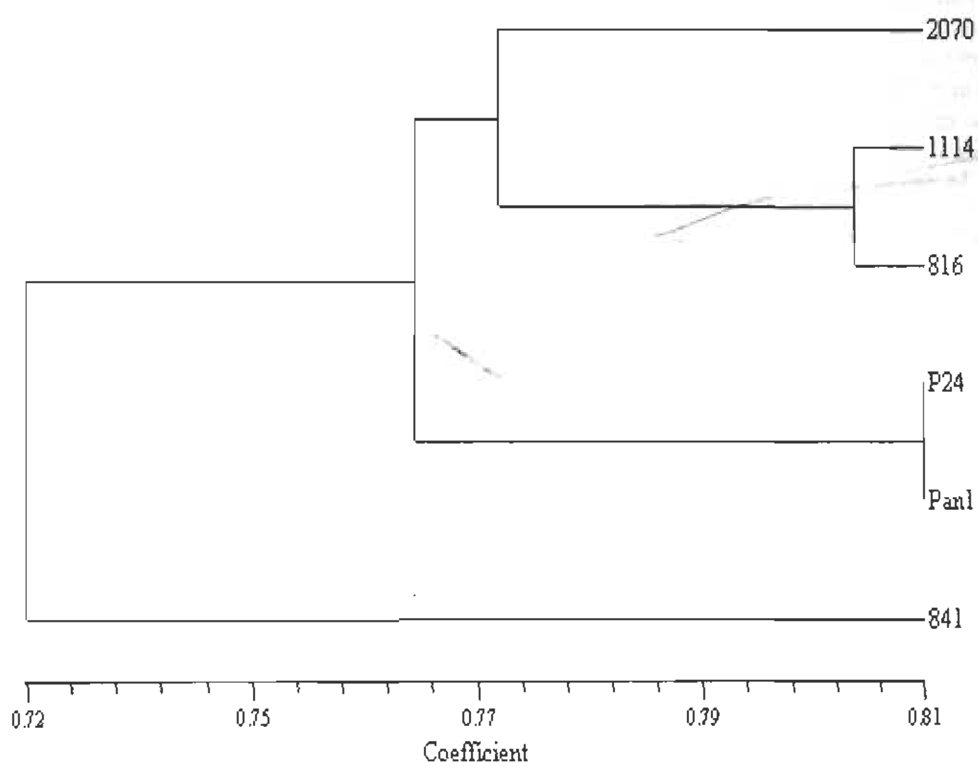

Fig. 23. Cluster analysis of black pepper *pollu* resistant lines (2070, 1114, 816, 841-resistant lines; P.24, Pan.1-susceptible lines)

oped against the protein as well as heat and glutaraldehyde treated *R. solanacearum* cells and their dilution end point was determined as 1:25,000. The antibodies were purified and their specificity and sensitivity were determined. Further western blot analysis showed that each of the developed antibody reacted with its own antigen besides reacting with the other two antigens used in the study.

Selective multiplication of **Ralstonia solanacearum**

In order to increase the detection limit of the assay, the serological assay was combined with bacteriological assay where the pathogen was selectively multiplied in the specific medium before the serological assay. Consequently, a selective medium was developed by testing various antibiotics against *R. solanacearum* and an enrichment medium was developed by incorporating those antibiotics, which did not affect the normal growth of the test pathogen.

Survival of **Ralstonia solanacearum** *in seed rhizomes*

Serological studies were carried out to detect the survival of *R. solanacearum* in ginger rhizomes stored at different temperatures for a period of 3 months. The results indicated that the rhizomes stored at 0 and 4° C sustained minimum bacterial population throughout the study period as observed by plate count and ELISA studies and virtually no disease incidence was observed under pot culture conditions.

Screening germplasm for bacterial wilt tolerance

Over 600 accessions of ginger were screened for bacterial wilt tolerance using simple soil inoculation method. Though all the accessions succumbed to the disease, some of the accessions (10) regenerated, indicating their tolerance to the disease, which was further confirmed using ELISA and these healthy rhizomes tested positive for *R. solanacearum.*

Induction of pathogenesis related proteins

Protein with molecular weight of 14 kDa was induced in root samples 4, 24 and 48 h after inoculation (HAI) with *R. solanacearum* and a protein with molecular weight of 38 kDa was induced in roots 4 HAI. No such induction was noticed in other tissues, which indicated that pathogenesis related proteins are induced in roots where the pathogen gains entry into the plant. Western blot showed the presence of chitinase and glucanase isoforms in ginger root tissues upon *R. solanacearum* inoculation.

Induction of novel proteins

When ginger was sprayed with protein preparations from *Boerhaavia diffusa*, three novel proteins of molecular weights 58.7, 105.4 and 98.0 kDa were induced in all the treatments, whereas it was absent in all the controls including the 0 h sample. It is interesting to note that the reduced level of expression of a major protein (67.9 kDa) in all the treated samples, which was very prominent in untreated calli. Two different proteins of molecular weights less than 53 kDa could be observed in ginger after 24 h of treatment with *Clerodendrum aculeatum* at concentrations of 200 and 250 ug/ml.

The protein preparation from *B. diffusa* and *C. aculeatum* (supplied by Dr. H. N. Verma) exhibited antibacterial activity against *R. solanacearum.*

Direct regeneration of ginger

An efficient and reproducible protocol for direct regeneration of plantlets through *in vitro* culture of pseudostem of ginger was achieved for the first time. Direct organogenesis in the form of shoots and root was observed in cultures containing BAP and NAA in all combinations, leading to multiple shoots in ginger.

Evaluation of antibiotics on calli

The minimum inhibitory concentration of various antibiotics was determined to screen transformed cells of ginger and cardamom. One of the accidental findings of screening antibiotics for preventing contamination in tissue culture was growth promotory activity of ampicillin. In ginger, ampicillin played a crucial role in organogenesis at lower concentrations.

Standardization of biolistics protocol for transformation

A protocol using biolistics was standardized with the plasmid pAHC 25 containing the GUS reporter gene encoding β-glucuronidase and the selectable marker BAR gene encoding phosphinothricin acetyl transferase driven by a maize ubiquitin promoter.

Standardization of Agrobacterium *mediated transformation*

The selected cardamom callus (infected with osmotin construct, pGV 2260) failed to regenerate. The screened callus was unaltered in the regeneration medium and did not show any sign of organogenesis. But the callus did not show any sign of cell death or difference in its colour. However, none of the ginger calli infected with this construct could survive even the first round of selection.

Transformation of ginger and cardamom for constitutive expression of glucanase and chitinase was done using vector pBZ100, driven by CaMV 35S promoter. Though ginger showed proliferation from the top of the callus placed on the selection medium, later it was found that the callus could not survive during subsequent exposure to the antibiotic in the next round of selection.

Purification of cardamom mosaic virus

A protocol for isolation and purification of cardamom mosaic virus (CaMV) from *katte* infected cardamom leaves was standardized. General protocol with borate buffer system (0.5 M) and a combination of antioxidants, sodium chloride (1.50 to 1.75%), PVP (2%), urea (0.5 M) and anionic detergents was more suitable to purify the virus from infected samples.

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, S. S. Veena, K. V. Ramana, Santhosh J. Eapen, V. Srinivasan and C. K. Thankamani*)

A field trial was laid out during 1999–2000 to identify the ideal combination of cultural practices for the management of *Phytophthora* foot rot disease involving maintenance of weed cover and clean cultivation, planting of two varieties of black pepper, application of organic and inorganic nutrients and chemical and biological control. The vines in the plot with clean cultivation recorded higher yield (1.544 kg/vine) than the plot with maintenance of weed cover (0.830 kg/vine). There was no significant difference in the nutrient status except potash, which was higher in the organic plot when compared to the inorganic plot.

The ongoing field trials on effect of planting varietal mixtures on the incidence of the disease were maintained and biocontrol agents and chemicals were applied in the respective plots. The populations of *Trichoderma* sp. and *Phytophthora* sp. were also monitored. Though there was positive baiting indicating the presence of the pathogen, there was no incidence of the disease in any of the treatments.

A study was conducted at Peruvannamuzhi to rejuvenate slow decline affected black pepper gardens by using five promising isolates of Plant Growth Promoting Rhizobacteria (PGPR). The physical condition of the vines was recorded at the start of the experiment and at fortnightly intervals. There was gradual reduction of yellowing of the vines and after 60 days most of the vines showed remission of yellowing (Table 63).

2. Utilization of *Piper colubrinum* Link and *Piper arboreum* as root stocks in the management of foot rot disease of black pepper

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

Four plots of 160 black pepper grafts (cleft, tongue and double rootstock grafts) on *Piper colubrinum* rootstocks were established in a low lying area with arecanut as standard at

Table 63. Effect of PGPRs on foliar yellowing of diseased black pepper vines

Treatment	Plants exhibiting yellowing (%)				
	0 DAT	15 DAT	30 DAT	45 DAT	60 DAT
IISR-6	34.6	26.6	13.3	8.9	9.1
IISR-51	47.8	30.0	9.5	4.5	2.3
IISR-151	34.9	15.9	4.4	2.2	2.3
IISR –853	28.5	19.9	13.2	4.7	0.0
IISR-859	37.8	30.5	12.4	5.7	2.3
Control	24.3	22.5	17.9	15.5	18.1
CD (P<0.05)	NS	NS	NS	NS	10.0

DAT = Days after treatment

Peruvannamuzhi. Grafts were also established in farmer's plots in Goa and Kerala (Kadangadu, Thrissur District).

The total yield of 690 grafts in a farmer's field at Peruvannamuzhi was 735 kg (dry) during the fifth year and no casualty or decline symptoms were observed (Fig. 24). Planting of six or seven rootstocks per standard was ideal to obtain a good canopy of scion to a height of 2 m within 1 year with good management and with an yield of 3 kg (green)/standard during the first year. Under water logged marshy situations, summer irrigation of grafts is not necessary as observed from the 5 year old garden at Peruvannamuzhi.

Observation of grafts of more than 5 years old in existing plots indicated over growth of scions but no cracks or splitting at the region of the union were observed. Similarly, no decline symptoms were observed in these grafts.

Analysis of berries obtained from various types of grafts for oleoresin, piperine and essential oil contents did not show variation, indicating no adverse effects due to grafting methods adopted.

Fig. 24. Black pepper vines grafted on *Piper colubrinum* rootstock

3. Biological control of diseases of spices

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

Mass multiplication of PGPRs

Mass multiplication of *Pseudomonas fluorescens* using inexpensive materials such as mature coconut water (0.1%) and molasses was standardized. The organism reached a population level of 10^{12} within 48 h.

An experiment was conducted using three types of coir pith namely, raw coir pith and coir pith composts I and II. *Trichoderma* inoculum that was produced by liquid fermentation using molasses as the medium in a fermenter was introduced into the coir pith compost in three different proportions, namely, 1:10, 1:20 and 1:40. The study was made in sterile and non-sterile conditions. The population of *T. harzianum* in these media was estimated immediately after inoculation and at 15 days interval up to 45 days.

The population of *T. harzianum* was reduced in unsterilized media except in the case of coir pith compost I (1:10) where it was stabilized at cfu 10^4. Sterilized coir pith composts I and II supported the growth of *T. harzianum* and showed an increase in population from 10^4 to 10^6 cfu per g of coir pith. In sterilized media, both commercial coir pith composts I and II supported the growth of *T. harzianum*. The population increased from 10^4 (9. 67 x 10^4) to 10^7 (4.5 x 10^7) cfu per g in sterilized coir pith compost I, that was inoculated in 1:10 proportion. Other proportions also showed increase in cfu from 10^4 to 10^6, in both the commercial coir pith composts. Raw coir pith did not support multiplication of *T. harzianum*, when compared to the other two media.

A new experiment to evaluate the efficacy of efficient strains of PGPRs and *Trichoderma harzianum* was initiated at Chelavoor.

Characterization of PGPRs

Fifty isolates of PGPRs were characterized based on carbon utilization and antibiotic resistance. The data was analysed using NTSYS software, which revealed the presence of 14 clusters based on carbon utilization and 22 clusters based on antibiotic resistance. Eleven of the isolates were identified as *P. fluorescens* based on their ability to utilize succinic acid.

4. Investigations on nematodes associated with spices

(*K. V. Ramana and Santhosh J. Eapen*)

Screening of ginger and turmeric germplasm

Twenty-four accessions of ginger and 59 accessions of turmeric were screened against root-knot nematode, *Meloidogyne incognita* among which of 4 each of ginger (Accs. 79, 197, 216 and 219) and turmeric accessions (Accs. 54, 56, 57 and 106) showed resistant reaction. Acc. 117, a root-knot nematode resistant ginger germplasm accession was approved for release as 'IISR Mahima' by the 21st State Seed Subcommittee, Kerala.

Organic amendments for nematode management

Studies carried out under microplot conditions indicated that incorporation of *Piper colubrinum* and *Strychnos nux-vomica* leaves in black pepper basins controlled nematodes and improved the yield of plants.

5. Biological control of nematodes of spices

(*Santhosh J. Eapen, K. V. Ramana and A. Kumar*)

Studies on PGPRs

Five isolates of rhizobacteria (IISR-522, IISR-528, IISR-658, IISR-853 and IISR-859) having dual nematicidal action (suppressing both *Radopholus similis* and *Meloidogyne incognita*) were short-listed from a collection of 291 isolates (Table 64).

Table 64. Promising isolates of PGPRs

Treatment	Nematode population/g root	
	Radopholus similis	*Meloidogyne incognita*
IISR-552	86.57	14.8
IISR-528	17.08	12.0
IISR-658	6.52	0.0
IISR-853	5.23	0.0
IISR-859	4.16	0.0
Nematode alone	1008.20	124.8
Absolute control	0.00	0.0

Twenty-five isolates of PGPRs were evaluated for their carbon utilization and antibiotic resistance. Many of them utilized succinic acid indicating that they belong to fluorescent pseudomonas group. Genomic DNA of these isolates was isolated through the CTAB-SDS method and a procedure for Rep-PCR finger printing was standardized.

Scaling up of promising isolates

Studies on mass multiplication of *Verticillium chlamydosporium* on solid substrates like rice bran, tapioca powder, decomposed coir compost and neem oil cake showed that rice bran was the best substrate (number of chlamydospores: 2.24×10^7). The synergistic effect of some plant extracts (having nematicidal effect) on mass multiplication of *V. chlamydosporium* was studied. Aqueous extracts of *Azadirachta indica* and *Chromoleana odorata* supported good growth and sporulation of *V. chlamydosporium*.

Sodium alginate and talc based formulations of rhizobacteria and *V. chlamydosporium* were prepared and are being evaluated for their shelf life. Additives like rice bran, ginger leaf powder, tea waste and tapioca powder were incorporated in these formulations and their impact on multiplication and survival of biocontrol agents is being studied.

Field trials with promising biocontrol agents

Field evaluation of promising fungal isolates namely, *Trichoderma harzianum* and *V. chlamydosporium* and the bacterium, *Pasteuria penetrans*, was continued for the fifth year. Black pepper vines treated with *V. chlamydosporium* followed by vines treated with phorate yielded higher than other treatments. The economics of biological control of nematodes of black pepper was worked out. Application of *V. chlamydosporium* resulted in a cost benefit ratio of 1: 7.1 in comparison with 1: 3.7 of phorate application and 1: 2.9 of *Trichoderma* application.

In another trial at Peruvannamuzhi, all the four fungal isolates namely, *V. chlamydosporium*, *T. harzianum*, *Paecilomyces lilacinus* and *Scopulariopsis* sp. significantly reduced foliar yellowing in black pepper vines.

Promising isolates of *V. chlamydosporium*, *T. harzianum*, *Fusarium oxysporum*, *Scopulariopsis* sp. and *P. lilacinus* were evaluated for their potential to suppress root-knot nematodes in ginger. Though none of them could significantly increase the yield of ginger, the highest yield was obtained with the application of *V. chlamydosporium* (Table 65).

Inducing variability in Verticillium *isolates*

Genomic DNA of three isolates of *V. chlamydosporium* and one isolate of *V. lecanii* was isolated.

Table 65. Evaluation of fungal bioagents on ginger in the field

Treatment	Height (cm)	No. of tillers	Fresh yield (kg/3x1 m bed)	Nematodes/g root
Control	74.98 a	9.39 a	5.15 bc	24.40 b
Verticillium chlamydosporium	73.99 a	9.32 a	5.83 c	1.95 a
Trichoderma harzianum	74.75 a	8.75 a	3.98 a	6.97 ab
Fusarium oxysporum	3.49 a	9.36 a	4.75 ab	23.67 b

Values with same letter designation are not significant according to DMRT at P<0.05

6. Bioecology and management of mealybugs infesting black pepper

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

Distribution

Surveys were conducted in black pepper growing tracts of Madikeri District in Karnataka to study the distribution of root mealybug, *Planococcus* sp. The areas surveyed included Hervanad, Napokulu, Boikeri, Horoor, Almatti, Valagunta, Suntikoppa, Akkatoor, Siddapur, Margoli, Polibetta, Mayamudi and Balale. Thirty nine black pepper gardens were surveyed out of which 13 gardens showed mild and 3 gardens medium level infestation by *Planococcus* sp.

Life history

The morphometrics of adults and crawlers of root mealybug were determined. The total life span from crawler to adults ranged from 32 to 36 days. The total number of eggs laid by females ranged from 19 to 197.

7. Biological control of insect pests of spices

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

Evaluation of entomopathogens against root mealybug

Four isolates of fungi belonging to the genera *Aureobasidium*, *Humicola*, *Paecilomyces* and *Scolecobasidium* collected from spice ecosystem were evaluated for their pathogenecity against root mealybug (*Planococcus* sp.) infesting black pepper in laboratory bioassays. However, none of the isolates were effective in preventing the multiplication of root mealybug after treatment.

Four isolates of entomopathogenic nematodes (*Heterorhabditis* sp.) were evaluated for their pathogenecity against root mealybug in laboratory bioassays. The study indicated that the isolates caused 10%–32% mortality of the pest.

Management of shoot borer

A commercial neem product (Nimbicidine) and neem oil were evaluated at 0.5%, 0.75% and 1.0% concentrations in the field at Peruvannmuzhi for the management of shoot borer (*Conogethes punctiferalis*) on ginger and turmeric 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–October was also maintained.

The trials indicated that spraying of Nimbicidine 1.0% and neem oil 1.0% were effective and was on par with malathion 0.1%. The percentage of shoots infested by the pest was significantly less in these treatments when compared to all other treatments including control. However, on turmeric, none of the treatments were effective in reducing the damage caused by the shoot borer.

Management of rhizome scale

Dried leaves of four plant species namely, *Chromoleana odorata*, *Glycosmis cochinchinensis*, *Melia composita* and *Strychnos nux-vomica* were evaluated as storage material for the management of rhizome scale (*Aspidiella hartii*) on ginger during storage after dipping the rhizomes in quinalphos 0.075%. The trials indicated that, storage of rhizomes in dried leaves of *S. nux-vomica* after dipping in quinalphos 0.075% was more effective for obtaining higher recovery of rhizomes and lesser incidence of rhizome scale.

8. Characterization of bioactive compounds with pesticide properties

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

Leaf extract from *Chromolaena odorata* (fungitoxic against *Phytophthora capsici*) was fractionated by column chromatography to isolate fungitoxic compounds. These fractions were further purified and two crystalline compounds were isolated from the active fractions. Spectral studies of the compounds are under progress.

Final Report

1. Isolation and identification of naturally occurring compounds against major pests and pathogens of black pepper

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

Objectives

The study was initiated with the objective of screening extracts of various plants for their pesticide properties against pests and pathogens of black pepper namely, *Phytophthora capsici*, root knot nematode and *pollu* beetle with a view to isolate and identify active principles in them and to evolve ecofriendly methods using botanicals for management of these pests and pathogens.

Preparation of extracts

Aqueous, hexane and methanol extracts of various plant species were prepared for evaluation of antifeedant, fungicidal and nematicidal properties.

Aqueous extract

50 g of the powdered plant material was extracted with hot water several times and the combined extract was concentrated to 50 ml and used in the bioassay.

Hexane and methanol extracts

Dried and powdered plant materials were successively extracted with hexane and methanol for 15 h in a Soxhlet apparatus and the extracts were separately concentrated to dryness and used in bioassays.

Essential oil

Essential oils were collected by hydrodistillation of fresh plant materials for 3 h. The oils were dried using anhydrous sodium sulphate and used in bioassays.

Screening for antifungal activity

The efficacy of plant extracts on mycelial growth of *Phytophthora capsici* was studied by poisoned food technique. The test extracts were mixed with 2% carrot agar to obtain concentrations ranging from 0.25% to 2.00% in the final volume of 60 ml of medium. This 60 ml medium was dispensed into four 9 cm petriplates. *P. capsici* from black pepper was cultured on carrot agar, 1 cm diameter mycelial discs were cut with cork borer and placed in the centre of each plate. In control sets, appropriate quantity of distilled water was used in place of plant extracts. The plates were incubated at 25°C (±1°C) and the growth of colony was measured after 72 h and 120 h after inoculation and per cent inhibition was calculated (Table 66).

Among the various extracts, essential oil from leaves of allspice showed promising fungitoxicity, followed by *C. odorata* leaf extract and *Z. rhetsa* seed oil. Allspice leaf oil completely inhibited mycelial growth of *P. capsici* at 0.5% whereas *C. odorata* leaf ex-

Table 66. Effect of aqueous plant extracts on mycelial growth of *Phytophthora capsici*

Extract	Conc. (%)	Inhibition of mycelial growth (%)*
Piper colubrinum leaf	2.00	83.8
	1.00	56.2
	0.50	34.3
	0.25	20.3
Lantana camera leaf	2.00	72.1
	1.00	68.2
	0.50	59.0
	0.25	52.6
Strychnos nux-vomica leaf	2.00	38.1
	1.00	18.4
	0.50	6.4
	0.25	0.0

(Table 66 continued next page)

(Table 66 continued from previous page)

Azadirachta indica leaf	2.00	75.5
	1.00	64.1
	0.50	38.2
	0.25	21.7
Chromolaena odorata leaf	2.00	100.0
	1.00	97.7
	0.50	82.0
	0.25	68.7
C. odorata root	2.00	100.0
	1.00	55.4
	0.50	24.7
	0.25	8.6
Zanthoxylum rhetsa seed oil	2 00	100.0
	1.00	62.0
	0.50	28.0
Annona squamosa seed - hexane extract	2.00	16.6
	1.00	11.6
	0.50	7.2
A. squamosa seed - methanol extract	2.00	41.5
	1.00	33.2
	0.50	17.2
Polyalthia longifolia leaf - hexane extract	2.00	65.7
	1.00	57.5
	0.50	46.0
P. longifolia leaf - methanol extract	2.00	66.0
	1.00	52.0
	0.50	35.5
Melia composita seeds - hexane extract	2.00	58.5
	1.00	46.8
	0.50	30.4
M. composita seeds - methanol extract	2.00	63.1
	1.00	25.1
	0.50	27.1
Allspice leaf oil	2.00	100.0
	1.00	100.0
	0.50	100.0
Eugenol	2.00	100.0
	1.00	100.0
	0.50	100.0

tract and Z. *rhetsa* seed oil inhibited mycelial growth at 2% concentration.

Isolation of antifungal principle in allspice leaf oil

The essential oil from allspice leaves caused complete inhibition of mycelial growth of *P. capsici* at concentrations ranging from 0.5% to 2.0%. Bioassay using the authentic sample of eugenol as well as fractions of the essential oil rich in eugenol caused the same fungitoxicity at the same concentrations. Hence, the fungitoxic principle in allspice leaves was identified as eugenol.

Antifungal activity of flavones

The effect of the two flavones isolated from *P. colubrinum* leaves namely, 7-methoxy 5, 4'-dihydroxy flavone (genkwanin) and 7-methoxy 5, 3', 4'-trihydroxy flavone (7-O-methyl luteolin) was tested on sporulation of *P. capsici in vitro*; sporulation was completely inhibited at 5000 ppm (Table 67).

Table 67. Effect of flavones from *Piper colubrinum* leaves on sporulation of *Phytophthora capsici*

Conc. (μg/ml)	Sporulation inhibition (%)*	
	Genkwanin	7-O-methyl luteolin
5000	100	100.0
500	52	66.0
50	0	7.2

*Mean of 3 replications

Isolation of active principles from Chromolaena odorata *leaves*

The dried and powdered leaves of C. *odorata* (100 g) were successively extracted with petroleum ether, chloroform and methanol for 15 h in each case in a soxhlet apparatus. Through bioassay guided fractionation by column chromatography, a fungitoxic compound was isolated.

Screening for nematicidal activity

The nematicidal activity of plant extracts was determined by immersion test method. Among the extracts, allspice leaf oil was the most promising, resulting in 100% mortality of test nematodes at 1320 ppm after 24 h. Complete mortality of test nematodes was obtained using methanol extract of *A. squamosa* seeds (2500 ppm), after 24 h of incubation. Aqueous leaf extract of *S. nux-vomica* and *P. colubrinum* exhibited 93.3% and 92.0% mortality of test nematodes, respectively. Aqueous leaf extracts of *L. camara*, *C. odorata*, *Z. rhetsa* seed oil, hexane and methanol extracts of *P. longifolia* leaves and *M. composita* seeds did not show significant nematicidal toxicity.

Isolation and identification of nematicidal principle from allspice

The major component in essential oil of allspice leaves was identified as eugenol by GC-analysis. The toxic effect of the oil as well as eugenol (at concentrations of 2640, 1320, 660, 330 and 165 μg/ml) was tested on second stage juveniles of *Meloidogyne incognita*. Both the oil and eugenol possessed the same nematicidal activity at identical concentrations. Hence the nematicidal principle in allspice was identified as eugenol (Table 68).

Isolation and identification of nematicidal principles from Piper colubrinum

Preliminary studies using aqueous leaf extracts indicated that the nematicidal activity resided in the nonpolar fraction of *P. colubrinum* leaves. But when the dried and powdered leaves (150 g) were extracted successively with petroleum ether, chloroform and water, the chloroform extract showed higher activity. Therefore the residue from chloroform extract was subjected to column chromatography over silica gel and further

Table 68. Nematicidal activity of allspice leaf oil and eugenol

Extract/Compound	Conc. (ppm)	Mortality over control after 24 h (%)*	Mortality over control after 48 h (%)*
Allspice leaf oil	2640	100.0	100.0
	1320	100.0	100.0
	660	70.6	99.2
	330	8.9	22.9
	165	5.2	16.0
Eugenol	2640	100.0	100.0
	1320	100.0	100.0
	660	73.6	100.0
	330	14.9	30.7
	165	9.5	14.1

*Mean of 3 replications

fractionated into four fractions namely, petroleum ether-ethyl acetate 9:1, petroleum ether-ethyl acetate 8:2, petroleum ether-ethyl acetate 7:3 and ethyl acetate, by elution with petroleum ether-ethyl acetate mixtures of increasing polarity. These fractions were concentrated to dryness and a part of each of these fractions was tested for nematicidal activity. The fractions eluted with petroleum ether-ethyl acetate 8:2 and petroleum ether-ethyl acetate 7:3 showed higher activity compared to others. Further purification of these two fractions yielded two crystalline compounds designated as genkwanin and 7-O-methyl luteolin, respectively. The effect of these fractions on second stage juveniles of *M. incognita* was studied (Table 69).

The nematicidal compounds isolated from *P. colubrinum* leaves were identified as 7-methoxy 5, 4'-dihydroxy flavone (genkwanin) and 7-methoxy 5, 3', 4'-trihydroxy flavone (7-O-methyl luteolin) by UV, IR, ^{1}HNMR, ^{13}CNMR and MS studies. Among the 2 compounds, 7-O-methyl luteolin showed higher nematicidal activity.

Screening plants for antifeedant activity on **pollu** *beetle*

In vitro antifeedant activity of plant extracts on *pollu* beetle (*Longitarsus nigripennis*) was tested by no-choice tests. Leaf discs of 1 cm size were cut from tender leaves of black pepper (*var.* Panniyur-1, a known susceptible variety) and dipped in various concen-

Table 69. Nematicidal toxicity of compounds from *Piper colubrinum* leaves

Concentration (ppm)	Mortality over control after 18 h (%)*		Suppression in hatching over control after 5 days (%)*	
	Genkwanin	7-O-Methyl luteolin	Genkwanin	7-O-Methyl luteolin
500	74	100	41.5	67.5
250	32	48	0.0	15.5
166	2	3	0.0	0.0

*Mean of 3 replications

trations of the extracts, air dried and placed in 100 ml beakers over moist filter paper for retaining humidity. Adult *pollu* beetles were collected from the field, starved for 12 h and five beetles were introduced into each beaker, the mouth of which was closed with a muslin cloth. The experiment was replicated four times. A control was maintained in which the leaf discs were dipped in distilled water. The leaf area fed by the beetles during a 24 h period was calculated by placing the leaf discs over a grid under a stereomicroscope (Table 70). The antifeedant index or per cent feeding deterrence was calculated by using the equation:

$$\frac{\text{Area fed in control} - \text{Area fed in treatment}}{\text{Area fed in control} + \text{Area fed in treatment}} \times 100$$

Table 70. Antifeedant activity of plant extracts on *pollu* beetle

Extract (1%)	Feeding deterrence (%)*
Annona squamosa seed (H)	100.0
A. squamosa seed (M)	100.0
Melia composita seed (H)	51.2
M. composita seed (M)	20.9
Polyalthia longifolia leaf (H)	55.8
P. longifolia leaf (M)	38.4
Zanthoxylum rhetsa fruit	0.0
Chromolaena odorata leaf (A)	34.0
Strychnos nux-vomica leaf (A)	64.7
Azadirachta indica leaf (A)	32.2
CD ($P<0.05$)	8.3

*24 h after treatment, average of 4 replications
H=Hexane extract; M=Methanol extract;
A=Aqueous extract

Among the various plant extracts, hexane and methanol extracts of *A. squamosa* seeds showed maximum feeding deterrence causing 100% deterrence (Fig. 25). These extracts also caused mortality of *pollu* beetle after 24 h. Aqueous leaf extract from *S. nux-vomica* resulted in 64.7% antifeedant acivity followed by hexane extracts from *P. longifolia* leaves and *M. composita* seeds. Other extracts showed negligible feeding deterrence.

Fig. 25. Antifeedant activity of *Annona squamosa* seed extracts on *pollu beetle* (left-treated; right-untreated)

Externally Funded Projects

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*)

Isolation of biocontrol organisms

Fluorescent pseudomonads and *Trichoderma* spp., were isolated from black pepper roots and rhizosphere soil collected from Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Sikkim and added to the Repository of Rhizobacteria which has 832 strains at present.

Mode of action

The antagonistic potential of bacterial isolates and *Trichoderma* spp. was evaluated by dual culture technique against *Phytophthora capsici*, the foot rot pathogen of black pepper. The mode of action of PGPRs in protecting black pepper from infection by *P. capsici* was studied in detail. The fluorescent pseudomonad strains, IISR-51 and IISR-13 significantly inhibited radial growth of *P. capsici*. These strains also produced inhibitory HCN, siderophores and antibiotics (pyoluteorin and pyrrolnitrin). The efficient strains of *Pseudomonas fluorescens* namely, IISR-13, IISR–51, IISR-8, ISR-11 and IISR-6 and *Trichoderma* isolates namely, P-26, P-12, GV-19, Tav-25 and Th-39 produced mycolytic enzymes such as β-1,3 glucanases, β-1,4 glucanases and lipases. The efficient strains were characterized based on their efficiency in utilization of different carbon sources, antibiotic sensitivity, and utilization of succinic acid. The efficient bacteria also solubilized phosphate and making it available for the plant. The introduced biocontrol bacteria in black pepper were endophytic as revealed by fluorescent microscopic observations.

The selected isolates of PGPRs were evaluated in the greenhouse 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, *Radopholus similis, Meloidogyne incognita* and *P. capsici* indicating their potential for the management diseases of black pepper in the nursery.

Shelf life

The shelf life of *Trichoderma* in various coir pith based substrates was tested and coir compost + sorghum was identified as the best carrier media with a shelf life of 75 days. Locally available agricultural wastes namely, coconut water and molasses (0.5%) were evaluated for multiplication of bacterial biocontrol agents and both the media were identified as economical carrier media; molasses supported a population of 10^{15} per ml and coconut water supported 10^{15} per ml in 32 h.

Compatibility

The efficient biocontrol agents of black pepper were tested against pathogens of ginger and cardamom to develop a biocontrol consortium in a multiple cropping system involving several spice crops. The biocontrol agents suppressed the disease in these crops and also promoted their growth. The combination of *T. harzianum* isolate, IISR-1369 and *P. fluorescens* strain, IISR-11 could improve the vigour of both black pepper and ginger plants. The same treatment combination resulted in maximum yield in ginger and cardamom. In field trials conducted with different combinations of fluorescent pseudomonads and *Trichoderma*, the best treatment was a combination of *Trichoderma* spp. (Is. nos. IISR-143 and IISR-369) and *P. fluorescens* (IISR-6) to decrease root rot disease besides increasing the yield. The two efficient biocontrol agents *P. fluorescens* (IISR-6) and *T. harzianum* (P-26) were individually efficient in protecting black pepper from root rot, compatible with each other and rhizosphere competent also.

X. Mega project: Economics, statistics and modelling

(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 predominantly cardamom growing districts of Idukki (Kerala) and Kodagu (Karnataka), to update the cost of cultivation data for major spice crops. The estimated cost of cultivation for cardamom during the crop year 2001–02 in Idukki and Kodagu districts was Rs. 213.97 and Rs. 222.17/kg, respectively.

Analysis of marketing system for spices

During the course of the survey, information on marketing aspects was also collected. More than 75% of the produce was marketed through auction centers and lot sizes less than 16 kg were not brought to the auction centre. More than 50% of the lots were less than 100 kg lots. Bodinayakanur (Madurai District, Tamil Nadu) was the major packing and forwarding centre for cardamom mainly because of lower labour wages (Rs. 22/day). About 40% of the produce was transported by train to domestic markets; road transport was cheaper (Rs. 3.50/km) than train (Rs. 6.40/km). Delhi and Kanpur were the major destinations for the produce from Bodinayakanur. Detailed analysis of seasonal variation in cardamom prices is being done based on the price obtained from auction centers.

Economics of spice production technologies

The cost of various spice production technologies was assessed (Table 71 to 76).

Digitization of database

Published secondary data were collected and used for populating the digital database on spice technology. Data on area, production, export, import and price for various spices were collected, refined and loaded into a database software for statistics. Database for 10 years was made available through the web site <www.spicestat.org> Commercial profile of all major spices was prepared and made available in the database.

2. Identification of appropriate prediction systems in spice crops

(K. N. Kurup and P. Rajeev)

Forecast of production of a crop facilitates decision making process with regard to its demand and availability (supply) and is an essential tool in formulating export-import policies. The annual production of commercially important spice crops exhibits unexplainable fluctuations and the project aims at developing forecast systems for a given crop and to assesses the extent to which it is disturbed by externalities.

The time series of annual production of three major spice crops namely, black pepper, ginger and turmeric were subjected to statistical analysis. Mathematical models like curve estimation, exponential smoothing and autoregressive moving average models (ARMA and ARIMA) were employed. It was established that curve fitting could be employed if prediction of a long-term trend is needed. But for making short-term predictions, exponential smoothing and ARIMA models are suitable. ARIMA models are

Table 71. Economics of cardamom cultivation in Kodagu District (Karnataka) (2001–02)

Particulars	Cost (Rs./acre)
Establishment cost (for 2 yrs)	
Labour	11,972.70
Planting materials	3000.00
Manures	6500.00
Fertilizers	2025.56
Pesticides	5944.50
Total establishment cost	29,442.76
Maintenance cost	
Labour	17,360.20
Compost	2837.50
Fertilizers	2250.72
Pesticides	3963.40
Drying charges	10,294.50
Interest on working capital @ 13% per annum	4037.66
Total maintenance cost	40,743.98
Estimated cost of cultivation	
Amortized value of initial investment @ 11% per annum	4999.46
Total maintenance cost per year	40,743.96
Total cost of production	45,743.44
Returns	
Average annual production/acre (dry capsule)	205.89
Gross returns @ Rs. 636.37/kg	1,31,022.21
Net returns	83,728.17
Cost of production/kg of dry capsules	**222.17**

preferable because of the feasibility of physically interpreting the parameters estimated.

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, Utpala Parthasarathy, K. Kandiannan and K. V. Saji)

The project was initiated during January 2003. The main objective of the project for the first year was to create digital maps for Western Ghats in general and Wyanad District in particular. Accordingly, soil maps for Kerala and Karnataka and water shed maps for Kerala were procured and the process of digitization initiated. An effort was made to identify land use and land suitability pattern for black pepper in Kerala making use of the database created for spices and the available digital data.

Externally Funded Projects

1. NATP: Integrated National Agricultural Resources Information System

(M. S. Madan, V. Srinivasan and K. S. Krishnamurthy)

Time series data were collected and sorted for entry into database. Software for stor-

Table 72. Cost of cultivation for rapid multiplication of rooted black pepper cuttings (bamboo method) (2003)

Particulars	Cost (Rs.)
Nonrecurring expenditure	
Clearing, leveling and providing drainage in nursery area	Available
Semi-permanent nursery structure of 24 m x 6 m size	97,000.00
Masonary charges including cost of cement	15,000.00
Shade net	12,000.00
Total	1,24,000.00
Recurring expenditure (once in 3 years)	
Bamboo 200 pieces @ Rs. 54 /piece	10,800.00
Arranging bamboo splits (10 mandays) @ Rs. 120 /day	1200.00
Mother vines 600 nos. @ Rs 6/vine	3600.00
FYM 260 cft @ Rs 20/cft	5200.00
Forest soil 650 cft @ 600/cft	3900.00
Sand 260 cft @ Rs. 9/cft	2340.00
Fumigation of potting mixture	1300.00
Coir dust 60 cft @ Rs. 2/cft	120.00
Preparation of rooting medium (240 cft) (6 man days)	720.00
Preparation of potting mixture (982 cft) (15 man days)	1800.00
Total	30,980.00
Recurring expenditure (every 3 years)	
Fertilizers	
Urea 29 kg @ Rs. 4.65 /kg	134.85
Super phosphate 29 kg @ Rs. 3.10/kg	89.90
Muriate of potash 15 kg @ Rs 4.35/ kg	65.25
Magnesium sulphate 7 kg @ Rs. 3.50 / kg	24.50
Cow dung slurry	100.00
Plant protection chemicals	
Ridomil 1 kg @ Rs. 1300 /kg	1300.00
Phorate 22 kg @ Rs. 50 /kg	1050.00
Quinalphos 1 lit @ Rs. 320/l	320.00
Copper oxychloride 10 kg @ 14.61 /kg	610.00
Charges for application of nutrient solution and pesticides	1950.00
Labour charges for tying vines, irrigation and maintenance	38,000.00
Cost of temporary shed (1000 capacity, 12 m x 6 m)	9500.00
Cost of shade net	9000.00
Total	62,144.50
Annuity value @ 11 %	26,315.00
Total cost of production	88,459.50
Cost of production/cutting	4.95

Table 73. Cost of cultivation for rapid multiplication of rooted black pepper cuttings (trench method) (2003)

Particulars	Quantity required	Unit cost (Rs)	Total cost (Rs)
Labour (man days)	4 nos.	80.00	320.00
Polybags	150 nos.	0.30	45.00
Farm yard manure	4 kg	2.00	8.00
Fytolan	30 g	0.23	6.90
Runner shoots (having 3 viable nodes)	50 nos.	2.50	125.00
Total cost per 150 nos./pit			504.00
Cost of production/cutting		**3.37**	

Table 74. Cost of production of vermi-compost

Items	Cost (Rs.)
Non-recrurring expenditure	
Clean thatched shed or shaded place	Available
Semi-permanent masonary tank (2.00 m x 1.00 m x 0.75 m size)	3200.00
Total	3200.00
Recurring expenditure during 1st year	
Earthworms	500.00
Cowdung (Rs./kg)	5.00
Vegetable waste	5.00
Labour cost (including masonary work)	6000.00
Total	6510.00
Total of items I & II	9710.00
Recurring expenditure during subsequent years	
Earthworms	Available
Cowdung	10.00
Vegetable waste	5.00
Labour	3000.00
Apportioned cost of initial investment @ 11%	1496.00
Total cost of production	4511.00
Cost of production/kg	**4.51**

Table 75. Cost of production of coir pith compost (100 kg)

Particulars	Quantity required	Cost/unit (Rs)	Total cost (Rs)
Coir pith	100 kg	1.50/kg	150
Urea	5 kg	4.80/kg	24
Spawn bottles	5 x 250 g	60/kg	75
Total man days	8	85/day	680
Total cost/100 kg			**929**

Table 76. Cost of production of *Trichoderma*

Particulars	Cost (Rs.)
Sorghum @ Rs. 5.30/kg including, loading, unloading and powdering	8.70
Transport	0.70
Autoclaving	4.50
Polypropylene bags (4 bags) @ Rs. 60/kg	0.80
Filling and autoclaving (50 p/bag)	2.00
Research time Rs 1500/30 days (producing 500/100 kg)	5.00
Depreciation-capital investment @10%	0.27
Institutional charges 40%	8.58
Quality maintenance (20% loss or contamination)	6.01
Packaging, escalation of cost of raw material	13.94
Total cost/kg	**50.50**

age of spice database in two parts was designed, modified and is being finalized. An interactive CD on 'Black Pepper Anthology' was prepared and sold through Agricultural Technology Information Centre. Two project personnels were trained in database management and Relational Database Management System and the facilities of the project were strengthened during the year with the purchase of equipments.

XI. Mega project: Extension and training

(Project leader: P. Rajeev)

1. Integrated disease management in black pepper-A study on technology diffusion and impact

(P. Rajeev)

The study is proposed to be undertaken in Wyanad District of Kerala. One hundred and forty beneficiary farmers from Wyanad who have procured the biocontrol formulation for the control of foot rot disease of black pepper from IISR during 1998 constitute the sample for the study. The procedure of stratified sampling based on number of black pepper vines subjected to the treatment by the respondents has been completed. The preparation of data collection tools like questionnaire and semi-structured interview schedule following a careful exercise of operational definition of variables to be studied has also been completed. The major variables defined under the study are knowledge domain of farmers, information seeking behaviour of farmers, diffusion pattern of technology, socio economic impact of technology and constraints in adoption of technology.

2. Training of research and extension workers

(P. Rajeev, M. S. Madan and T. K. Jacob)

Institute training programmes

The institute conducted four regular training programmes for field extension functionaries of agricultural and horticultural departments and research workers of other ICAR institutes and state agricultural universities, in which 32 trainees participated. The modules for these programmes were prepared based on the technologies developed by the institute. The topics covered included production technology, nursery management and post harvest technology. Two short term training programmes on bioinformatics sponsored by Department of Biotechnology were also organized in which 21 trainees participated.

Off-campus seminars

Scientists of the institute were deputed to deliver lectures in farmers and extension seminars. The institute participated in 16 programmes organized by various institutions like Central Plantation Crops Research Institute, Kasaragod; Indian Council of Agriculture Research Complex, Goa; Indian Agricultural and Farmers Congress, Meerut and Kerala State Department of Agriculture.

Project work by students

As a part of human resource development functions of the institute, short term research projects were carried out by MSc student research scholars from various universities. These projects were effectively integrated in to the research work already being carried out at the institute in the disciplines of biotechnology, microbiology, plant pathology, entomology, economics, biochemistry and computer applications. During 2002–03, 67 students carried out these research projects fetching a revenue of Rs. 3,02,500/- to the institute.

Externally Funded Projects

1. NATP: Agricultural Technology Information Centre

(P. Rajeev)

The Agricultural Technology Information

Centre (ATIC) facilitated direct sale of planting material of improved varieties of spices from the main campus as well as experimental farm and 75,440 rooted cuttings of black pepper and 722 of *Piper chaba* were sold. Pamphlets (1505), folders (629), technical bulletins (80) and institute publications (56) were also sold. A CD on 'Black Pepper Anthology' was produced and 36 nos. were sold. Soil (12) and manure samples (3 nos.) were also tested. Nine hundred and ninty-six farmers were benefited by advisory services. Two *kisan melas* were also organized.

2. **DBT: Distributed Information** Sub-centre

(Santhosh J. Eapen)

Research

Various databases and softwares were developed.

Databases

- An online database (http://www.iisr.org/bioinformatics/project/index.htm) on plant chitinases.
- An enzyme database of phenylalanine ammonia lyases (http://www.iisr.org/bioinformatics/ project/index.htm).
- A database of chemical compounds and metabolic pathways in cardamom volatile oil (http://www.iisr.org/bioinformatics/project/cardamom/index.htm).
- A database of *Curcuma* species including their characteristics and identification.
- A database of nutmeg germplasm collections at the institute.
- A bibliographic database, JOSAC, which is a cumulative index of the first 10 volumes of Journal of Spices and Aromatic Plants, was brought out on CDs.

Softwares

- Restalyzer: A software to find out the restriction sites in a nucleotide sequence.
- Translator: A software to predict the aminoacid sequence from nucleotide sequences.
- *Ralstonia* Biovars: An expert system for biovar characterization of *Ralstonia solanacearum*, the causal organism of bacterial wilt of ginger (http://www.iisr.org/ bioinformatics/project/ralstonia/index.htm).

Web page

A web page on *pollu* beetle, a major insect pest of black pepper was developed (http://www.iisr.org/bioinformatics/project/beetle/index.htm).

Website

The IISR website (www.iisr.org), designed by the Bioinformatics Centre, was frequently updated and maintained. The website is very popular and is being listed by major search engines. It also provides access to various databases developed by DISC and links to different related sites. A web enabled intranet information hub (SPICENET) having interactive user-friendly menus, links and pointers was developed and launched for the benefit of the institute staff.

Training

Two training programmes, 'Computer skills for biologists' and 'Introduction to bioinformatics' were organized for in-house and external participants. Several informal training programmes in computer applications were also conducted for the benefit of institute staff.

Infrastructure

- E-lab: The centre has set up a separate e-lab with independent nodes that provide round the clock access to various databases, softwares, bioinformatics tools and e-journals.
- Library resources: The centre has assembled various library resources like reference books (14), journals (7), CD-ROMs (3), on-line access to e-journals (2) and databases (1).

Traineeship and studentship

The centre has appointed two trainees to develop an exclusive web resource on *Phytophthora*, the most important fungal pathogen of horticultural crops. Studentships were awarded to four M.Sc. students doing their project work at the institute.

3. NATP: Prioritization, Monitoring and Evaluation

(*K. V. Ramana and M. S. Madan*)

The Prioritization, Monitoring and Evaluation (PME) Cell of the institute started functioning during 2002 and was formally inaugurated by Dr. Mruthyunjaya, Director, National Centre for Agricultural Economics and Policy Research, New Delhi on 18 January 2003 with the objectives of prioritization of institute research programmes, tracking of current resources allocations, project monitoring and evaluation and research impact analysis. The specific objectives of the project at the institute include, benchmark study of NATPs and identification of important technologies from NATPs for study of impact of technologies.

Collection of bench mark information and case studies of seven NATPs and an equal number of non-NATPs in progress at the institute was completed and the data from the study is being analysed.

Technologies Assessed and Transferred

High yielding black pepper varieties

Based on 7 years performance at Tata Tea Ltd, Valparai (3000 ft MSL, Tamil Nadu) and 2 years performance at Peruvannamuzhi, and quality analysis, three black pepper lines were recommended for release. These lines were superior over the controls for more than one character at one or both locations. Trials laid out at farmers plots in four northern districts of Kerala also indicated the superiority of the new lines.

Coll. 1041

The mean fresh yield per vine was 5.17 kg (row trial) and 1.61 kg (large block trial) as compared to 3.23 kg (row trail) and 1.45 kg (large block trial) of Panniyur-1 (control). Coll. 1041 also had high dry recovery (35%), bulk density (582 g) and essential oil (3.2%) when compared to Panniyur-1 at Valparai. The yield and bulk density of Coll. 1041 was superior to KS-27 (control) at Peruvannamuzhi. The line also showed field tolerance to disease at Valparai and Peruvannamuzhi.

HP-105

The mean fresh yield per vine was 6.14 kg (row trial) and 1.42 kg (large block trial) as compared to 3.23 kg (row trial) and 1.45 kg (large block trial) of Panniyur-1 (control) at Valparai. HP-105 also had higher dry recovery (32%), bulk density (582 g) and essential oil (3.2%) compared to Panniyur-1 (control).

HP-813

The mean yield per vine was only 2.78 kg in row trial whereas in large block trial it was 2.06 kg/vine against 3.23 kg (row trial) and 1.45 kg (large block trial) of Panniyur-1 (control). At Peruvannamuzhi, the yield (2.4 kg/vine) of this line was more or less equal to that of KS-27 (control). The hybrid was characterized by superior quality. Both at Valparai and Peruvannamuzhi, HP-813 recorded a high percentage of oleoresin (11.7% at Valparai compared to 9.4% of Panniyur-1 (control) and 14.6% at Peruvannamuzhi compared to 10.6% of KS-27 (control). The essential oil content of the line also surpassed the control at both the locations. The bulk density (612 g) and dry recovery (34%) were also superior to Panniyur-1 (control) at Valaparai.

Plant growth promoting rhizobacteria for black pepper

The plant growth promoting rhizobacteria (PGPR) isolate IISR-6, was an efficient colonizer of black pepper roots and enhanced growth of the plant and also suppressed root rot caused by *Phytophthora capsici*. This isolate was also compatible with the biocontrol agent *Trichoderma harzianum* (IISR-1369). When inoculated together, about 40% enhanced growth in black pepper was recorded. The isolate could be used for growth enhancement and disease suppression in black pepper plants both in the nursery and field. For large scale multiplication of PGPRs, agricultural and industrial wastes/by-products such as coconut water and molasses were suitable. The population of PGPRs increased to 10^{15} cfu within 32 h in coconut water and 48 h in molasses 0.5%.

Coconut coir pith as a carrier medium for biocontrol agents

Coconut coir pith, which is a commonly available agricultural waste, could be used effectively for mass multiplication of *T. harzianum*. Fresh coconut coir pith does not support the growth of biocontrol agents and the initial decomposition of coir pith could be done by adding urea and inoculating with *Pleurotus platypus*. Decomposed coir pith is sterilized by autoclaving and subsequently used as carrier medium for biocontrol agents. The coir compost commercially available could also be used after adjusting the pH to near neutral (6.5–7.0) by mixing coir compost with rock phosphate at 10:1 or lime/wood ash at 20:1 and incubating for 7 days. The population of biocontrol agents increases from 10^4 cfu to 10^7 cfu within 10 days. If the coir compost is added with coconut water, the population increases to this level within 5 days. In sterilized coir pith, these population levels can be maintained for 50 days. The biocontrol agents multiplied in liquid broth could be mixed in sterile coir compost and applied to the soil to control soil-bome diseases of spice crops.

Education and Training

Post graduate studies

Ph.D

K. P. M. Dhamayanthi. Studies on chilli pepper (*Capsicum annuum* L.). I. Transfer of certain specific agronomically desirable characters into popular chilli cultivars. II. Induction of mutations and autopolyploidy. III. Assessment of phylogenetic relationships by seed protein analysis. IV. Induction of haploids. Bharathiar University, Coimbatore.

K. Kandiannan. Influence of varieties, times of planting, spacings and nitrogen levels on growth, yield and quality, crop-weather and growth simulation modelling and yield forecast in turmeric. Tamil Nadu Agricultural University, Coimbatore.

Anu Augustine. Selection of promising lines, production of somaclones and their utilization in paprika (*Capsicum annuum* L.). University of Calicut, Calicut.

M.V.Sc

S. Ravi. Calcium and phosphorous requirement in indigenous layer ducks, Kerala Agricultural University, Thrissur.

M.Sc projects

Sixty-seven students from various universities undertook their M.Sc project work in Biotechnology, Bioinformatics, Biochemistry, Chemistry, Microbiology, Plant Pathology and Economics under the guidance of scientists of the institute.

Training programmes attended by staff

Modelling Growth and Yield of Crops, Indian Agricultural Research Institute, New Delhi, 27 March–17 April 2002 (K. S. Krishnamurthy).

Methodology of Impact Analysis of KVK's Programme Activities, Indian Institute of Horticultural Research, Bangalore, 12–13 April 2002 (P. S. Manoj).

High Performance Thin Layer Chromatography, Anchrom Laboratory, Mumbai, 22–26 April 2002 (T. John Zachariah).

Watershed Planning under Watershed Technology (Mission Mode) Project of NATP, Central Water Conservation Research and Training Institute, Research Centre, Udhagamandalam, 8–17 May 2002 (S. J. Ankegowda).

Emerging Trends in the Production Technology of Tropical Fruit Crops, Tamil Nadu Agriculture University, Coimbatore, 3–12 July 2002 (P. S. Manoj).

Training Technology, Indian Institute of Horticultural Research, Bangalore, 30 July–2 August 2002 (K. M. Prakash).

Information Technology in Agriculture, National Academy for Agriculture Research Management, Hyderabad, 31 July–20 August 2002 (K. V. Saji).

Frontiers in Ecofriendly Management of Crop Pests and Diseases and Utility of Biofertilizers in Improving Soil Fertility, Mahatma Phule

Krishi Vidyapeeth, Maharashtra, 5 August–4 September 2002 (A. Kumar).

Sustainable Production from Agricultural Watersheds in Hills, Vivekananda Parvatiya Krishi Anusandhan Sansthan, Uttaranchal, 21 August–10 September 2002 (K. Kandiannan).

Molecular Marker Application in Plant Breeding, Indian Agriculture Research Institute, New Delhi, 26 September–5 October 2002 (K. P. M. Dhamayanthi).

Use of Isotopes and Radiations in Soil-Plant Relationships, National Research Laboratory, Indian Agricultural Research Institute, New Delhi, 3 October–1 November 2002 (K. P. M. Dhamayanthi, K. N. Shiva).

Introduction to Bioinformatics, Indian Institute of Spices Research, Calicut, 20–22 November 2002 (B. Chempakam, P. A. Mathew).

Vigilance Awareness for ICAR Institutes in Southern Region, Central Tuber Crops Research Institute, Thiruvananthapuram, 18–20 December 2002 (T. John Zachariah, V. L. Jacob).

Molecular Biological Techniques for Horticultural Crops, Indian Institute of Spices Research, Calicut and Central Plantation Crops Research Institute, Kasaragod, 27 December 2002–4 January 2003 (B. Chempakam, J. Rema, R. Suseela Bhai, K. S. Krishnamurthy, K. V. Saji).

Training on Financial Management, National Institute of Financial Management, Faridabad, 6–17 January 2003 (V. L. Jacob).

Training on HRD Leadership Skills, Central Marine Fisheries Research Institute, Research Centre, Tuticorin, 20–25 January 2003 (Johny A. Kallupurackal).

Advanced Course on Web Designing, National Academy of Agricultural Research Management, Hyderabad, 24–29 March 2003 (Johny A. Kallupurackal).

Training programmes organized by the institute

Computer Skills for Biologists, 4–6 September 2002.

Introduction to Bioinformatics, 20–22 November 2002.

Molecular Biological Techniques for Horticultural Crops, 27 December 2002–4 Jan 2003.

Spices Production Technology, 13–15 January 2003.

On-farm Processing of Spices, 22–24 January 2003.

Orientation Training for Supporting Staff, 20–23 February 2003.

National Informatics Centre on Spices

The library of the institute under the National Informatics Centre on Spices was set up to 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.

At present, the library has a collection of 3800 books, 2621 bound volumes of journals, 2562 reprints, 756 technical reports and 96 theses. The library is subscribing to 31 foreign journals and 64 Indian Journals in addition to CABCDs and AGRISCDs. The new additions added to the library during the year include 180 books, 29 reprints, 71 technical reports and 20 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. The National Agricultural Technology Project allotted a sum of Rs. 3.25 lakhs for strengthening the library facilities and a computer, laser printer and new CABCDs were procured.

Awards and Recognitions

V. A. Parthasarathy, P. M. Kumaran, M. J. Ratnambal, Anitha Karun, V. Niral, V. Arunachallam and Anuradha Upadhyay. ICAR Award for Team Research for the Biennium 1999–2000.

A. K. Sadanandan, V. Srinivasan and S. Hamza. Best Poster Paper Award, for the paper 'Effect of integrated plant nutrient management system on yield and quality of Indian spices' presented at the XVII World Congress of Soil Science, Bangkok, Thailand, 14–21 August 2002.

C. K. Thankamani and P. K. Ashokan. Best Poster Paper Award, for the paper 'Influence of drip irrigation on yield and moisture extraction pattern of bush pepper grown in coconut garden' presented at the National Symposium on Soil and Water Conservation Measures and Sustainable Land Use Systems with Special Reference to Western Ghat Region, Goa, 16–17 November 2002.

K. M. Maya, T. John Zachariah and B. Krishnamoorthy. Alapati Prasada Rao Award for Best Research Paper (Poster), for the paper 'Lycopene and volatile oil constituents-Changes during storage of mace (*Myristica fragrans*) powder' presented at the National Seminar on Strategies for Increasing Production and Export of Spices, Calicut, 24–26 October 2003.

K. Nirmal Babu. Technical Expert, International Atomic Energy Agency, Vienna, sponsored project on 'Radiation induced mutations in black pepper' at Department of Agriculture, Sri Lanka, 9–13 December 2002.

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, Thiruvanathapuram.	Research collaboration in post harvest technology.
Central Plantation Crops Research Institute, Kasaragod.	Research collaboration in post harvest technology.
Centre for Water Resources Development and Management, Calicut.	Research collaboration in biosynthesis of pigments and translocation of pesticides.
Rajiv Gandhi Centre for Biotechnology, Thiruvanathapuram.	Research collaboration in molecular markers.
Kerala Agricultural University, Thrissur.	Research collaboration in biotechnological approaches for improvement of spices and evaluation of tissue cultured plants; Centre for Post Graduate studies.
University of Calicut, Calicut.	Centre for Post Graduate studies; MOU for teaching M.Sc. Biotechnology and M.Sc. Plantation Development students.
Bharathiar University, Coimbatore	Centre for Post Graduate studies.
Nagarjuna University, Nagarjunasagar.	Centre for Post Graduate studies; MOU for teaching and training MSc Biotechnology students.
Spices Board, Kochi.	Training programmes.
Craigmore Plantations, Nilgiris.	Evaluating black pepper lines for high altitudes.
Tata Tea Ltd., Valparai.	Evaluating black pepper lines for high altitudes.
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) with its headquarters at Calicut is one among the 80 coordinated research projects under the ICAR. The AICRPS was started in 1971 as a combined project on spices and cashew and was bifurcated into two separate projects one each for spices and cashew in 1985. The mandate of the AICRPS is to increase area, production and productivity of spices in the country through:

- Evolving high yielding varieties with quality attributes, tolerant/resistant to pests and diseases for various agro-ecological situations
- Standardizing agro-techniques for spice crops under different agro-climatic conditions
- Evolving cost effective and efficient pest and disease management practices
- Working as interface between SAUs, IISR and ICAR.

At present, 19 centres spread over 15 states based at 15 agricultural universities are functioning under AICRPS, in addition to 8 co-operating/voluntary centres. The total staff strength of AICRPS is 83, which includes 51 scientists and 32 technical/auxiliary staff. About 115 research projects are in progress at various centers under the AICRPS on 12 spice crops (black pepper, cardamom, ginger, turmeric, clove, nutmeg, cinnamon, corriander, cumin, fennel, fenugreek and large cardamom).

Crop improvement

The genetic resources of spice crops at various centres were strengthened and evaluation of germplasm for various parameters was carried out. At present, the germplasm of AICRPS centres consist of black pepper-461, cardamom-331, ginger-547, turmeric-1221, tree spices-137 and seed spices-3631. Promising germplasm accessions were identified and short-listed. New comparative yield trials (CVTs) were initiated in addition to existing CVTs. The lines DH-205, DG-234 (coriander), HF-107, HF-116 (fennel) and HM-372, HM-376, HM-444 (fenugreek) were identified as promising and included in CVTs. Promising cardamom genotypes in open pollinated (OP) seedling progenies (D-237, CL-730, 8-4-D11) and other superior clones (CL-692 and 7-24-D11) were identified at Mudigere. Cardamom lines tolerant to drought (CL-746, P-6, D-237 and CL-746) were also identified. The black pepper Acc. 239 was identified as a promising line at Sirsi. The ginger varieties, V_3S_1-8 and V_1E_8-2 and turmeric varieties, PTS-59, PTS-43 and PTS-55 were promising at Pottangi and are in the process of being released. The exotic line of coriander, EC-232666 was identified as promising (for leaf yield) for commercial cultivation.

Crop management

Fertilizer and irrigation requirements of black pepper-arecanut mixed cropping system were standardized at Sirsi. Studies at

Mudigere revealed the influence of micronutrients (boron and molybdenum) on yield of cardamom. Package of practices for ginger and turmeric were standardized at Chintapalli. A fertilizer package, including application of biofertilizers was standardized for clove and nutmeg at Yercaud. In seed spices, biofertilizers (*Azospirillum*) were found to influence the yield. The yield and quality of coriander and fennel increased by application of the micronutrients, Zn, Fe, Mn and Cu.

Crop protection

A package for the management of *Phytophthora* foot rot disease of black pepper was developed and recommended at Panniyur and Sirsi. Surveys for the incidence of diseases and pests in black pepper indicated the occurrence of anthracnose disease and leaf gall thrips in Idukki District, Kerala. In black pepper, two sprays of either monocrotophos (0.05%) or dimethoate (0.05%) at fortnightly intervals after harvest of berries was effective in reducing mussel scale (*Lepidosaphes piperis*) at high ranges of Idukki District. A low cost technology for mass multiplication of *Trichoderma* sp. for field application was developed at Sirsi. Rotting of ginger rhizomes in storage can be checked by storing the rhizomes treated with Dithane M-45 + Bavistin (5 g + 3 g/kg of seed) in pits with sand in layers. Control measures for wilt disease in coriander and rhizome rot in ginger were evolved by various centres. Surveys conducted in Bihar established the severity of stemgall disease in coriander.

Krishi Vigyan Kendra

Training programmes

The Krishi Vigyan Kendra (KVK) conducted 78 training programmes on various subjects during the year in which 2767 persons participated (Table 77).

Front line demonstrations

Coir pith composting and its utilization for banana

Ten demonstrations (0.1 acre each) were conducted at Poozhithode on composting of coir pith and its utilization for banana. Good quality compost was produced within 45 days. The compost when applied to rainfed banana (*var.* Nendran) @ 3 kg/plant exhibited enhanced growth compared to control receiving ordinary cultural practices.

Cultivation of coleus

Five demonstrations (0.05 acre each) of cultivation of high yielding variety (Sreedhara) of coleus were carried out. Nucleus planting material was multiplied and supplied to the selected beneficiaries during April 2002. The nursery was raised during the second week of April, and planting in the main field was taken up during second week of June. The crop was harvested during January–February 2003 (Table 78).

Integrated pig farming

A demonstration on raising of Yorkshire pigs was initiated at Chakkittapara with the objective of familiarizing the technique of integrated pig farming with other farming systems, to increase the production performance of piglets and farm income.

On-farm trials

Cultivation of capsicum

Two trials were laid out at Peruvannamuzhi (0.1 acre each) to evaluate the feasibility of cultivating capsicum (California Wonder, Indra and Arka Mohini) in Kozhikode District. Even though 80% of the seeds germinated, the seedlings were damaged by wilt in the nursery even after adopting recommended control measures against the disease. Very few seedlings survived and these

Table 77. Training programmes conducted by KVK during 2002–03

Category	No. of courses	No. of participants			No. of SC/ST participants
		Male	Female	Total	
Farmers	59	944	103	1976	106
Rural youth	14	296	194	490	76
Extension functionaries	5	95	24	119	-
Total	78	1335	1250	2585	182

Table 78. Economics of cultivation of coleus (*var.* Sreedhara)

Yield (kg/ha)	Local check		Improved variety		Increase		Effective gain
	C	R	C	R	C	R	(Rs.)
11,850	66,260	1,02,430	68,750	1,12,575	2,490	10,145	7,655

C= Cost of cultivation (Rs./ha); R = Returns (Rs./ha)

plants yielded on an average of only one fruit per plant. The preliminary study showed that the crop is not suitable for growing in this locality.

Efficacy of urea solution for wound healing

Accidental injury and subsequent maggot infection is the most common ailment of livestock requiring long duration treatment involving manpower and expenditure. A trial to evaluate the efficacy of urea solution (10% and 15%) for wound healing in dairy cattle was initiated and is under progress.

Demonstration units

Demonstration units on medicinal plants, model homestead garden, improved varieties in black pepper, model arecanut seed garden and cashew scion bank were maintained.

Revolving fund

Quality planting materials of various crops were produced and sold under this programme (Table 79).

Plant and Animal Health Centre

The KVK operates a Plant and Animal Health Centre offering various services to farmers. An artificial insemination facility is available at the centre to upgrade the genetic stock of livestock. The centre offers consultation, treatment and doorstep services with a nominal fee. The centre also provides vaccination facility and organises animal health camps in association with the State Animal Husbandry Department. The various activities taken up by the centre during the period is as follows:

- Consultancy/advisory/ home services 432
- Artificial insemination 292
- Farmers' study tour programmes 4
- Animal health campaigns/ infertility camps 8
- Vaccination of poultry birds and animals 841
- Quiz contest programme 1
- Seminars 5

Table 79. Production of planting materials

Crop	Variety	No. of plants sold
Arecanut seedlings	Mohitnagar, SAS-1	2589
Allspice seedlings	Elite lines	721
Bush pepper plants	Sreekara, Subhakara, Panchami, Pournami, Panniyur-1	184
Garcinia grafts	Elite lines	890
Mango grafts	Bennet Alphonso, Kalepady	39
Piper chaba rooted cuttings	Elite line	7371

An amount of Rs. 4,35,481/- lakhs was realized through sale of planting materials and activities of the Plant and Animal Health Centre.

Other extension activities

The KVK participated in various exhibitions and *kisan melas* (Fig. 26):

Fig. 26. Inaugration of *kisan mela* by Dr. R. K. Samanta, Zonal Coordinator, KVK

Thrissur Pooram, 5 April–20 May 2002, Thrissur.

National Consultative Meeting for Accelerated Production and Export of Spices, Directorate of Arecanut and Spices Development, 29–30 May 2002, Calicut.

Anniversary of Sunni Markaz, 8–13 October 2002, Calicut.

Agricultural Fair and Exhibition, Kerala Agricultural University, 19–20 October 2002, Ambalavayal.

National Seminar on Increasing Production and Export of Spices, Indian Institute of Spices Research, 24–26 October 2002, Calicut.

Calicut Flower Show 2003, 30 January–5 February 2003, Calicut.

Collaborative activities

The KVK associated with NGO's such as Centre for Overall Development (COD), Vikas Volunteer Vahini Club (VVV) and Indian Farmer's Movement (INFAM) in various activities. The training programmes imparted by the KVK helped some of the beneficiaries to begin small-scale employment units with follow up assistance from KVK (Fig. 27).

A 1 month training programme was organized for 20 women of self help groups (SHG's) on bamboo handicrafts making in association with COD, Thamarassery and Uravu, Wyanad. The members after completing the training programme started a small scale production unit at Peruvannamuzhi and generated an income of about Rs. 10,000/- through sale of handicrafts. Eleven farmers from Chakkittapara attended a training programme on vermicomposting and all of them started verniculture units. Presently, 20 SHG's in Chakkittapara are practising vermiculture as a small scale enterprise for meeting their own demand and also to sell the surplus compost. A SHG has sold 60 kg of earthworms, valued at Rs. 30,000/- through the assistance of KVK. A goatary unit was started at Muthukad with 15 goats as a consequence of training programme conducted at KVK. Two unemployed youth trained under KVK are engaged in production and sale of grafts, layers and cuttings of various fruit and spice plants under the revolving fund and they earn about Rs. 50,000/- each per year.

Fig. 27. A training programme organized by KVK in progress

Publications

Books and chapters in books

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On-going Research 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. Biotech. V (813): Large scale multiplication of released varieties of black pepper through somatic embryogenesis and genetic fidelity testing
5. Gen. X (813): Breeding cardamom for high yield and resistance to *katte* disease
6. Gen. XIV (813): Cytogenetics and reproductive biology of major spices
7. 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 modelling in black pepper

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

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. X (813): Investigation on vein clearing virus of small cardamom
4. Path. XI (813) : Studies on bacterial wilt of ginger
5. Path. XIV (813) : Studies on fungal and viral diseases of ginger
6. Path. XV (813): Investigations on viral and fungal diseases of vanilla

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. Nema. III (813) : Investigations on nematodes associated with spices
5. Biocontrol I.3 (813): Biological control of nematodes of spices
6. Ent. XI (813): Bioecology and management of mealybugs infesting black pepper
7. Biocontrol I.2 (813): Biological control of insect pests of spices

1. Org. Chem. II (813): Characterization of bioactive compounds with pesticide properties

X. Mega project: Economics, statistics and modelling

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. V (813): Integrated disease management in black pepepr–A study on technology diffusion and impact
2. 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. Characterization of nutmeg germplasm for quality
3. Elucidation of biosynthetic pathways of curcumin in turmeric
4. National Network Project on *Phytophthora* Diseases of Horticultural Crops

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. Field evaluation of tissue cultured plants of spices and assessment of their genetic stability using molecular markers
5. Immunoserological approaches to pathogen detection and use of defence proteins in disease management in plantation crops-ginger and cardamom
6. Compatibility, stability and potential of biocontrol consortium on suppression of *Phytophthora* foot rot of black pepper and their conservation
7. Distributed Information Sub-Centre

National Agricultural Technology Project

1. Collection, characterization and conservation of spices genetic resources
2. Molecular characterization and preparation of molecular maps in black pepper
3. Development and evaluation of soil-water conservation measures and land use systems for sustainable crop production in Western Ghats of Coastal Region
4. Integrated technologies for value addition and post harvest management in palms, spices and tropical tuber crops
5. Value addition and quality enhancement of selected spices
6. Integrated National Agricultural Resources Information System
7. Agricultural Technology Information Centre

Department of Agriculture and Cooperation

1. Centrally Sponsored Scheme-Integrated Programme for Development of Spices

Government of Kerala

1. Technology mission on black pepper

Emeritus Scientist Scheme

1. Plant nutrient management strategy for breaking black pepper yield plateau and quality upgradation.

Consultancy and Commercialization of Technologies

The Consultancy Processing Cell processed various consultancies relating to analysis of micro, macro and trace elements in soil, plant, water and serum samples; estimation of *Trichoderma* population and estimation of phenol and tanin in plant samples. The technology for large scale multiplication of *Trichoderma harzianum* was sold to four new entrepreneurs; four entrepreneurs also renewed the agreement. *In vitro* conservation facility was extended to Arya Vaidya Sala, Kottakkal, for conservation of medicinal plants for 3 months. The Consultancy Processing Cell earned Rs. 1,34,000/- through these consultancy services. The institute also earned Rs. 3,02,500/- through M.Sc. project work programmes of post graduate students of various universities.

Institute Management Committee

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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, Thrissur.	Member
Senior Finance and Accounts Officer, Central Marine Fisheries Research Institute, Kochi.	Member
Dr. M. N. Venugopal, Head in Charge, Cardamom Research Centre, Indian Institute of Spices Research, Appangala.	Member
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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 Finance and Accounts Officer, Indian Institute of Spices Research, Calicut.	Member
Assistant Administrative Officer, Indian Institute of Spices Research, Calicut.	Member Secretary

Quinquennial Review Team

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Dr. M. Balasubramanian	: Member
Dr. T. C. Jain	: Member
Dr. N. M. Nair	: Member
Dr. Anupam Verma	: Member
Dr. Koshy John	: Member
Dr. V. A. Parthasarathy	: Member
Dr. M. Anandaraj	: Member Secretary

The Quinquennial Review Team (QRT) reviewed the work done at the institute and also under the All India Coordinated Research Project on Spices during the period 1 January 1997 to 31 December 2001. The members of the QRT visited various laboratories of the institute at Calicut, experimental plots at Peruvannamuzhi and also a few centres of AICRPS and had discussions with scientists (Fig. 28). The report of the QRT is under preparation.

Fig. 28. Members of the QRT at Peruvannamuzhi Farm

Research Advisory Committee

Dr. K. V. Ahamed Bavappa	: Chairman
Prof. V. Arunachalam	: Member
Dr. C. K. George	: Member
Dr. R. K. Sharma	: Member
Mr. L. Venkataratnam	: Member
Dr. Y. R. Sarma	: Member
Dr. K. V. Ramana	: Member Secretary

The Research Advisory Committee meeting was held during 17–18 May 2002 at Calicut and the following recommendations were made.

General recommendations

1. All the existing projects should be regrouped under mega projects with specific focus and objectives with time targets to achieve the goals.
2. Special focus is needed to promote group activity and intra and inter-discipline collaboration at project and experiment execution levels in various disciplines.
3. Only significant results need be emphasized and presented in RAC meetings to enable fruitful discussions on the same.
4. Presentation of results, particularly of yield and quality performance must be in proper format mentioning years of evaluation, design of experimentation and appropriate statistical parameters like SE, CV, CD, etc.
5. Cross-cutting themes need be recognized and presented in the discipline that has a major share to avoid duplication. Scientists from concerned disciplines can interact during discussions.
6. All the equipments in the institute should be shared by scientists as a common facility and duplication of purchase, particularly major and costly equipments should be avoided during X Plan.
7. A meeting between IISR and NGOs should be organized to discuss the feasibility of transfer of technologies developed at the institute.

Crop Improvement and Biotechnology

1. Both quality and yield parameters have to be given due importance while recommending a variety for release.
2. In addition to yield, varietal development should also take care of drought tolerance, resistance to pests and diseases, quality and amenability to post harvest processing.
3. Selection should be the major breeding strategy and new avenues of breeding strategies such as convergent crosses should be taken up.
4. One or two major crops should be identified and specific breeding strategies formulated and tested.
5. Germplasm characterization must be complemented by appropriate utiliza-

tion not only as new varieties but also as parents in hybridization programmes.

6. Molecular characterization of germplasm should be matched and integrated with morphometric characterization.

7. Integration of molecular breeding with conventional methods is important and markers linked to potential traits have to be identified.

8. The performance of ginger microtubers has to be tested on a large scale in farmer's fields and biocontrol agents are to be incorporated along with microtubers.

Crop Production and Post Harvest Technology

1. The influence of agrochemical practices on quality of spices has to be studied.

2. The quality of promising cassia lines is to be compared with that of cassia from Vietnam and Indonesia available in the market.

3. Research on developing processing and post harvest technologies should be reoriented based on consumer and market requirements.

4. Studies may be initiated on pharmacological properties of spices in collaboration with other institutions.

5. Emphasis should be given to develop on-farm processing techniques for producing value added products.

6. Due emphasis should be given for mechanization of various farm operations such as irrigation, harvesting, grinding, cleaning, drying, etc.

7. To determine agronomic requirements, trials should be undertaken with the more important of the released varieties.

8. Studies on nutrient requirement of different spices under monocropping and multiple cropping systems are to be studied.

9. Studies on organic farming are to be conducted adopting prescribed standards.

Crop Protection

1. Developing *Phytophthora* foot rot resistant varieties in black pepper should be given high priority.

2. The effect of organic farming in management of diseases of spice crops should be studied.

3. Studies on aflatoxins in spices may be initiated.

4. The economics of management of nematodes should be worked out in all the experimental trials.

5. Suitable storage materials including paddy husk may be evaluated for preventing infestation of rhizome scale during storage of ginger.

Social Sciences

1. Cost benefit analysis of promising technologies should be updated.

2. Technology dissemination through mass media may be given higher priority for wider coverage of clientele.

3. A database has to be developed on the extent of coverage of new varieties and technologies developed by the institute and the benefit accrued to the farming community.

Staff Research Council

The XVI Staff Research Council (SRC) meeting of the institute was held during 4–6 April 2002 at Calicut (Fig. 29). The Inaugural Session was held on the forenoon of 4 April 2002. Dr. Y. R. Sarma, Director, delivered the inaugural address and highlighted the significant achievements of the institute during the past year.

The Staff Research Council meeting had four technical sessions that were chaired by Dr. K. V. Ahamed Bavappa, Former FAO Consultant; Dr. V. Rajagopal, Director, Central Plantation Crops Research Institute, Kasaragod; Dr. H. Hameed Khan, Project Coordinator, All India Coordinated Research Project on Palms, Kasaragod; and Dr. P. Balasubramanian, Professor of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The progress of work in 42 institute projects were presented and discussed and the technical programme for the ensuing year approved in these sessions. Four new projects were also presented and approved. Five projects were recommended to be closed during the year.

Fig. 29. SRC meeting in progress

The Plenary Session was held during the afternoon of 6 April 2002 and was chaired by Dr. K. V. Ahamed Bavappa. Dr. Y. R. Sarma, welcomed the members and invitees to the Plenary Session. The 'Research Highlights 2001–02' was released by Dr. K. V. Ahamed Bavappa. The significant achievements and the future thrust areas of research were presented by the heads of divisions/section. Recommendations for management of root mealybug and root knot nematode infesting black pepper, for extension agencies, were presented and approved. The meeting came to a close with a vote of thanks by Dr. J. Rema, Secretary, Research Council.

The mid-term review of research projects was held during 8–9 January 2003 at Calicut. The meeting was chaired by Dr. V. A. Parthasarathy, Director, and was co-chaired by various heads of divisions. Based on the reccomendations of the Research Advisory Committee meeting held during March 2002, the 42 research projects in progress at the institute were grouped under 11 mega projects and project leaders were identified for the same. Two new projects were also presented and approved.

Participation of Scientists in Meetings

Brain Storming Session on Chillies/Paprika, Indian Institute of Spices Research, Calicut, 8 April 2002 (All scientists).

30th Town Official Language Implementation Committee Meeting, Calicut, 18 April 2002 (B. Krishnamoorthy).

Town Official Language Implementation Sub-committee Meeting, Calicut, 29 April 2002 (B. Krishnamoorthy).

Hindi Workshop, Town Official Language Implementation Committee, Calicut, 22 May 2002 (K. N. Shiva).

Seminar on Investment Opportunities in Herbal Spices, Coimbatore, 23 May 2002 (B. Sasikumar).

National Consultative Meeting on Accelerated Production and Export of Spices, Calicut, 29–30 May 2002 (B. Chempakam, B. Krishnamoorthy, T. John Zachariah, B. Sasikumar, J. Rema, C. K. Thankamani, K. N. Shiva).

Seminar on Official Language, Calicut, 19 June 2002 (B. Krishnamoorthy).

Review Meeting of All India Radio Programme, Mannakavu, 20 June 2002 (P. A. Mathew).

Annual Research Council Meeting, Indian Cardamom Research Institute, Myladumpura, 24–27 June 2002 (M. N. Venugopal).

Workshop on Strengthening Food Safety and Quality Systems in India through the work of National Codex Committee, Kochi, 1–2 July 2002 (T. John Zachariah).

Research-Extension-Farmers Interface, Malappuram, 5 July 2002 (T. John Zachariah, P. Rajeev).

Research-Extension-Farmers Interface, Ambalavayal, 5 August 2002 (T. John Zachariah, B. Sasikumar).

Workshop on Biodiversity Conservation, Hyderabad, 12–14 August 2002 (K. N. Shiva).

Workshop on Development of Integrated Horticulture, Mizoram, 30–31 August 2002 (M. N. Venugopal).

Workshop on Integrated Technologies for Value Addition and Post Harvest Management in Palms, Spices and Tropical Tuber Crops, Thiruvananthapuram, 5 September 2002 (T. John Zachariah).

21st State Seed Sub-committee Meeting, Central Tuber Crops Research Institute, Thiruvananthapuram, 18 September 2002 (B. Sasikumar, J. Rema).

Workshop on Use of Irradiation-Sterilization through Ionization Process, Kochi, 20 September 2002. (T. John Zachariah).

Workshop on National Biodiversity, Strategy and Action Plan for Kerala, Thrissur, 23–24 September 2002 (B. Sasikumar, J. Rema).

District Panchayat Meeting for Improvement of State Seed Farms, Calicut, 1 October 2002 (P. A. Mathew).

Workshop on Coffee Based Cropping System, 5 October 2002, Balehonnur (M. N. Venugopal).

Zonal Review Workshop of Transfer of Technologies Projects, Dindigul, 7–9 October 2002 (P. Rajeev, P. S. Manoj).

National Horticulture Conference 2002, New Delhi, 18–19 October 2002 (S. Devasahayam).

National Seminar on Strategies for Increasing Production and Export of Spices, Calicut, 24–26 October 2002 (All scientists).

31st Meeting of Calicut Town Official Language Implementation Committee, Calicut, 6 November 2002 (B. Krishnamoorthy).

International Conference on Vegetables, Bangalore, 11–14 November 2002 (D. Prasath).

XXIV Zonal Workshop of Research and Extension Advisory Council (NARP Northern Zone), Kasaragod, 12 November 2002 (B. Krishnamoorthy).

National Symposium on Soil and Water Conservation Research and Sustainable Land Use System with Special Reference to Western Ghat Region, Goa, 15–17 November 2002 (S. J. Ankegowda, C. K. Thankamani).

Workshop on Radiation Processing of Spices, Kochi, 22 November 2002 (B. Chempakam).

Geographical Indication on Spices-WTO Taskforce, Sub-committee Meeting, Calicut, 24 November 2002 (B. Sasikumar).

Discussion Meeting on Applied Chemical Ecology: Implications of Induced Resistance and Transgenics in Insect-Plant Interactions, Chennai, 30 November 2002 (S. Devasahayam).

PLACROSYM-XV, Mysore, 10–13 December 2002 (M. N. Venugopal, T. John Zachariah).

Seminar on Spices Production Technology, Goa, 20–21 December 2002 (J. Rema, V. Srinivasan).

Second International Agronomy Congress, Balancing Food and Environmental Security-A Continuing Challenge, New Delhi, 26–30 December 2002 (C. K. Thankamani).

Joint Meeting of the Steering Committee of State Biodiversity Strategy and Action Plan for Kerala and State Biodiversity Board, Thiruvananthapuram, 16 January 2003 (B. Sasikumar).

Hazard Analysis for Critical Control Points for Chilli Safety Management, Guntur, 4–5 February 2003 (T. John Zachariah, N. K. Leela).

Brain Storming Session on Eco-tourism and Event Management, Calicut, 5–6 February 2003 (V. A. Parthasarathy, P. A. Mathew, K. V. Saji, K. N. Shiva).

Hindi Workshop on Noting and Drafting, Calicut, 14 February 2003 (K. N. Shiva).

Annual Biotechnology Information System Co-ordinators Meeting, Kolkata, 21–22 February 2003 (Santhosh J. Eapen).

National Workshop on Human Resource Development Strategies for Organizational Effectiveness of NARS, Hyderabad, 11–12 March 2003 (V. A. Parthasarathy, S. Devasahayam).

One Day Farmers' Seminar, Calicut, 15 March 2003 (B. Sasikumar).

Regional Seminar on Spices, Virajpet, 20 March 2003 (M. N. Venugopal).

Seminar on Vanilla and Black Pepper Cultivation, Thadiyankudisai, 20 March 2003 (K. Kandiannan).

Patent Awareness Workshop, Thrissur, 21 March 2003 (B. Sasikumar).

Meeting on Empowerment of Women, Kochi, 27 March 2003 (B. Chempakam, R. Suseela Bhai).

Meetings Organized by the Institute

Braining Storming Session on Chillies/Paprika, Calicut, 8 April 2002 (Fig. 30).

National Seminar on Strategies for Increasing Production and Export of Spices, Calicut, 24–26 October 2002.

Fig. 30. Dr. G. Kalloo, Deputy Director General (Horticulture and Crop Sciences), ICAR, New Delhi, inaugurating the Brainstorming Session on Chillies/Paprika

Radio Talks

Cardamom planting and after care, All India Radio, Madikeri, 25 June 2002 (S. J. Ankegowda).

Nutritive value of common spices, All India Radio, Calicut, 7 August 2002 (T. John Zachariah).

Cultivation of bush nutmeg graft, All India Radio, Calicut, 24 August 2002 (P. A. Mathew).

Propagation techniques and plant protection in pepper, All India Radio, Madikeri, 5 November 2002 (M. N. Venugopal).

Medicinally important spices suited to commercial cultivation, All India Radio, Calicut, 28 December 2002 (B. Sasikumar).

Nematodes in spices-problems and control, All India Radio, Calicut, 29 January 2003 (Santhosh J. Eapen).

Quality improvement of black and white pepper, All India Radio, Calicut, 29 January 2003 (K. M. Prakash).

Agronomic practices for better yield in cardamom, All India Radio, Madikeri, 10 February 2003 (S. J. Ankegowda).

Harvesting and processing of pepper and cardamom to maintain quality, All India Radio, Madikeri, 23 February 2003 (M. N. Venugopal).

Lectures Delivered by Scientists

Post harvest processing of spices, Central Plantation Crops Research Institute, Kasaragod, 17 April 2002 (T. John Zachariah).

Vanilla cultivation, Farmers Meet, Chittoor, 6 May 2002 (R. Suseela Bhai).

Vanilla and pepper cultivation, Training for Agricultural Officers, Kunnamangalam, 20 May 2002 (B. Sasikumar).

Pests and diseases of black pepper and vanilla, Training for Agricultural Officers, Kunnamangalam, 21 May 2002 (Santhosh J. Eapen).

Vanilla and turmeric-Investment opportunities in herbal spices, Entrepreneurs and Farmer's Meet, Chennai, 23 May 2002 (B. Sasikumar).

Vanilla diseases, Training for Agricultural Officers, Tenhipalam, 4 June 2002 (R. Suseela Bhai).

Black pepper-Pests and diseases, Training for Agricultural Extension Officers, Balussery, 5 June 2002 (Santhosh J. Eapen).

Vanilla cultivation, Farmers Meet, Agali, 27 June 2002 (R. Suseela Bhai).

Vanilla production, Training for Agricultural Officers, Manjeri, 29 June 2002 (B. Sasikumar).

Processing for white pepper and other value-added products from pepper, Research-Extension-Farmers Interface, Malappuram, 5 July 2002 (T. John Zachariah).

Pepper production management, Research-Extension-Farmers Interface, Malappuram, 5 July 2002 (P. Rajeev).

Improved technologies on spices production management, Research-Extension-Farmers Interface, Calicut, 6 July 2002 (P. Rajeev).

Quality improvement and value-addition in black pepper, Research-Extension-Farmers Interface, Ambalavayal, 5 August 2002 (T. John Zachariah).

Biotechnology in agriculture, Research-Extension-Farmers-Interface, Ambalavayal, 5 August 2002 (B. Sasikumar).

Vanilla cultivation, Farmer's Day Celebration, Cheekode, 17 August 2002 (B. Sasikumar).

Cardamom cultivation, Farmer's Meeting-Quality Improvement in Cardamom, Galibedu, 29 August 2002 (S. J. Ankegowda).

Bioinformatics and its applications, St. Joseph's College, Calicut, 9 September 2002 (Santhosh J. Eapen).

Cardamom cultivation, Farmer's Meeting - Quality Improvement in Vanilla and Cardamom, Bettageri, 12 September 2002 (S. J. Ankegowda).

New trends in cultivation of pepper and cardamom, Training for Agricultural Extension Officers, Sirsi, 19 September 2002 (M. N. Venugopal).

Crop Improvement of black pepper, ginger and turmeric, National Training on Advances of Spices Production Technology and Medicinal Crops, Thrissur, 24 September 2002 (B. Sasikumar).

Cardamom, black pepper and vanilla cultivation, Control of Berry Borer Programme for Progressive Farmers, Mutharmudi, 3 October 2002 (S. J. Ankegowda).

Vanilla production, Farmer's Training Programme, Kodiathur, 7 October 2002 (B. Sasikumar).

Mixed crops of cardamom and pepper in arecanut plantations, Farmer's Training Programme, Sirsi, 17 October 2002 (M. N. Venugopal).

Potential of spices in mixed crop conditions, Training for Agricultural Officers, Shimoga, 18 October 2002 (M. N. Venugopal).

An introduction to bioinformatics, The Zamorins Guruvayurappan College, Calicut, 28 October 2002 (Santhosh J. Eapen).

Quality improvement of black pepper, Training for Horticultural and Agricultural Officers, Gudalur, 6 November 2002 (K. Kandiannan).

Potential of spices in arecanut and coconut gardens, Training for Agricultural Officers, H. D. Kote, 5 December 2002 (M. N. Venugopal).

Cardamom and pepper cultivation, Farmer's Training Programme, Maragodu, 11 December 2002 (S. J. Ankegowda).

High value spices in arecanut and coconut plantations, Training for Agricultural Officers, Bramhavar, 20 December 2002 (M. N. Venugopal).

Spices-Methods in processing and value addition, Farmer's Training Programme, Thamarassery, Calicut, 20 January 2003 (T. John Zachariah).

Value addition in black pepper, State Level Training to Extension Personnel, Calicut, 21 January 2003 (T. John Zachariah).

Post harvest processing and value addition in spices, Training for Agricultural Assistants, Calicut, 30 January 2003 (T. John Zachariah).

Production technology of pepper, Training for Progressive Farmers, Saklespur, 6 February 2003 (M. N. Venugopal).

Pest and disease management in vanilla, Training for Progressive Farmers, Appangala, 10 February 2003 (M. N. Venugopal).

Production of polyclonal antiserum, Winter School, Tamil Nadu Agricultural University, 15 February 2003 (A. Ishwara Bhat).

Serological methods for the detection of viruses, Winter School, Tamil Nadu Agricultural University, 15 February 2003 (A. Ishwara Bhat).

Production technology of cardamom, Farmer's Training Programme, Gonikoppal, 18 February 2003 (M. N. Venugopal).

Cardamom cultivation, Bhagamandala Vevasaya Seva Sahakara Bank and Farmer's Club Meeting, Bhagamandala, 8 March 2003 (S. J. Ankegowda).

Product diversification and value addition in spices, Farmer's Training Programme, Ambalavayal, 15 March 2003 (T. John Zachariah).

Production management in vanilla, Research-Extension-Farmers Interface, Calicut, 17 March 2003 (P. Rajeev).

Prospects of spices as intercrop in coffee plantations, Farmer's Training Programme, Kargunda, 18 March 2003 (M. N. Venugopal).

Promotion of vanilla cultivation, Farmer's Meet, Mampad, 19 March 2003 (R. Suseela Bhai).

Varieties, pest and disease management and post harvest processing of pepper, Training Programme for Progressive Farmers, Appangala, 19 March 2003 (M. N. Venugopal).

Production technology of pepper, Training Programme for Progressive Farmers, Virajpet, 20 March 2003 (M. N. Venugopal).

New varieties and improved practices for black pepper production, Seminar on Vanilla and Black Pepper Cultivation, Thadiyankudisai, 20 March 2003 (K. Kandiannan).

Option for organic cultivation of cardamom, pepper and ginger, Training for Agricultural and Extension Officers, Napoklu, 25 March 2003 (M. N. Venugopal).

Spices cultivation, Coffee Berry Borer Control Programme, Bhagamandala, 26 March 2003 (S. J. Ankegowda).

Cardamom cultivation, Coffee Berry Borer Control Programme, Yevakapadi, 28 March 2003 (S. J. Ankegowda).

Distinguished Visitors

Dr. K. V. Ahamed Bavappa, Former FAO Consultant and Former Director, Central Plantation Crops Research Institute, Kasaragod.

Dr. D. K. Arora, Director, National Bureau of Agriculturally Important Microorganisms, New Delhi.

Dr. E. V. V. Bhaskara Rao (Late), Director, National Research Centre for Cashew, Puttur.

Mr. T. K. Chandrashekhar, General Manager, Bharat Sanchar Nigam Limited, Calicut.

Dr. B. S. Dhankar, Assistant Director General (PC), Indian Council of Agricultural Research, New Delhi.

Dr. O. P. Dubey, Assistant Director General (PP), Indian Council of Agricultural Research, New Delhi.

Dr. Javed Iqbal, Director, Regional Research Laboratory, Thiruvananthapuram.

Mr. Joseph Thekke Kuruvinal, Chief Judicial Magistrate, Calicut.

Dr. G. Kalloo, Deputy Director General (Horticulture and Crop Sciences), Indian Council of Agricultural Research, New Delhi.

Dr. S. P. S. Kanuja, Director, Central Institute for Medicinal and Aromatic Plants, Lucknow.

Dr. Khadrekar, Former Vice Chancellor, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Mr. Lawrence V. Fernandes, Member, Governing Body, Indian Council of Agricultural Research, New Delhi.

Mr. E. T. Lukose, Commissioner of Income Tax, Calicut.

Dr. T. Madhan Mohan, Director, Department of Biotechnology, New Delhi.

Dr. K. P. G. Menon, Executive Director, International Pepper Community, Jakarta, Indonesia.

Dr. Mruthyunjaya, Director, National Centre for Agricultural Economics and Policy Research, New Delhi.

Dr. R. Naidu, Director (Research), Central Coffee Research Institute, Balehonnur.

Dr. M. K. Nair, Former Director, Central Plantation Crops Research Institute, Kasaragod.

Dr. K. V. Peter, Vice Chancellor, Kerala Agricultural University, Thrissur.

Dr. Raghava V. Thampan, Director, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Dr. R. B. Rai, Director, Central Arid Zone Research Institute, Port Blair.

Dr. V. Rajagopal, Director, Central Plantation Crops Research Institute, Kasaragod.

Dr. M. R. Sethuraj, Former Director, Rubber Research Institute of India, Kottayam.

Dr. S. Thamburaj, Former Dean, Tamil Nadu Agricultural University, Coimbatore.

Dr. Tamil Selvan, Director, Directorate of Arecanut and Spices Development, Calicut.

Dr. J. Thomas, Director (Research), Indian Cardamom Research Institute, Myladumpara.

Empowerment of Women

A Women's Cell has been set up at the institute to cater to the welfare and solve grievances of women who form about 20% of the staff of 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) located at IISR Farm, Peruvannamuzhi (Fig. 31). These programmes were conducted in association with Departments of Agriculture and Animal Husbandry and voluntary organizations such as Centre for Overall Development (COD), Vikas Voluntary Vahini (VVV) clubs and Indian Farmer's Movement (INFAM). These agencies are sponsoring numerous Self Help Groups (SHG), which are presently engaged in several income generating activities. The KVK conducted 32 self-employment generating training programmes for these group members in which 712 members participated. The programmes covered various aspects of agriculture, horticulture, animal husbandry, fruit processing and handicrafts as indicated below:

Fig. 31. Training programme in bamboo crafts for women entrepreneurs

1. **Post harvest technology and cottage industries**

a. Fruit processing and preservation (223 participants, 9 training programmes).

b. Production of oyster mushroom (57 participants, 2 training programmes).

c. Extraction and processing of banana fibre (33 participants, 1 training programme).

d. Handicrafts making from bamboo (50 participants, 2 training programmes).

e. Artificial flower making (19 participants, 1 training programme).

2. **Seed production and establishment of nurseries**

a. Production and utilization of vermicompost (134 participants, 6 training programmes).

b. Production of bush pepper (26 participants, 2 training programmes).

c. Cultivation of medicinal plants (29 participants, 1 training programme).

d. Cultivation of coleus (19 participants, 1 training programme).

3. **Rearing and care of poultry and livestock**

a. Management of milch cows (14 participants, 1 training programme).

b. Production and management of goats (14 participants, 1 training programme).

c. Management of goat diseases (26 participants, 2 training programmes).

d. Management of contagious diseases in cattle (35 participants, 1 training programme).

e. Control of external parasites in livestock by indigenous practices (33 participants, 1 training programme).

As a result of the various training programmes, several self-employment units were started in vermicomposting, bamboo handicraft making, poultry and goatary, and food processing, earning additional income through sale of products. The KVK also conducted a frontline demonstration programme on 'Introduction of a HYV of coleus namely, Sreedhara' in five women farmers fields.

The international Women's Day was celebrated on 8 March 2003 at the institute. Singing and elocution competitions were also held for the staff of the institute during the celebrations.

Other activities

National Science Day

The National Science Day celebrations were organized during 24–28 February 2003. The inaugural function was held on 24 February 2003 during which Mr. K. N. Kurup, Head, Social Sciences Section, delivered a talk on 'Science in ancient India'. A elocution competition on the topic 'Human cloning-good or bad' and a quiz competition in English and Malayalam was held for the staff of the institute and school children, during the celebrations. The valedictory function was held on 28 February 2003 and Dr. K. P. Aravindan, Associate Professor, Medical College, Calicut, delivered a talk on 'Human genome project' and also distributed prizes to winners of various competitions (Fig. 32).

Fig. 32. Dr. K. P. Aravindan, Associate Professor, Medical College, Calicut, delivering the valedictory address during the National Science Day celebrations

Vigilance Awareness Week

The Vigilance Awareness Week was observed during 31October–6 November 2002. The week long programme was inaugurated by Mr. T. K. Chandrasekhar, General Manager, Bharat Sanchar Nigam Limited, Calicut, on 31 October 2002 with the administration of a pledge by Dr. V. A. Parthasarathy, Director. Various competitions such as essay writing, elocution and slogan writing were held in English and Malayalam for the staff of the institute and school children. The topic for the elocution contest was 'Vigilance awareness-transparency in public life'. The valedictory function was held on 6 November 2002 during which Mr. E. T. Lukose, Commissioner of Income Tax, Calicut, delivered the valedictory address and distributed prizes to winners of various competitions.

Hindi Fortnight

The Hindi Fortnight was celebrated during 14–29 September 2002. The celebrations were inaugurated by Dr. V. A. Parthasarathy, Director, on 14 September 2002. Staff members of the institute took an oath during the inaugural function for using Hindi in day to day work. Various competitions such as essay writing, noting and drafting and calligraphy were conducted for staff members. Dr. V. A. Parthasarathy, Director, distributed prizes to winners of various competitions during the valedictory function held on 29 September 2002.

Kisan Divas

Kisan Divas (Farmers Day) was celebrated on 23 December 2002 and about 100 farmers participated in the function organized at IISR Farm, Peruvannamuzhi. Ms. M. K. Nalini, District Panchayat President, Calicut, who was the Chief Guest, highlighted the need for transfer of latest technologies to farmers for improving production of spices and other commodities. Thirteen farmers were honoured in the function for their contribution to farming of various spices (Fig. 33).

Recreation Club

The Recreation Club of the institute organized competitions on singing of national anthem, national song and patriotic songs during the Republic Day celebrations on 26 January 2003. Farewell functions were also arranged to staff members who left the institute during the year.

Fig 33. A progressive farmer being honoured during *kisan divas*

Personnel

Headquarters, Calicut

Managerial

Dr. Y. R. Sarma, Director (Retired on 31 May 2002)

Dr. V. A. Parthasarathy, Director (Joined on 17 August 2002)

Scientific

Dr. P. N. Ravindran, Project Coordinator (Retired on 30 April 2002)

Dr. K. V. Ramana, Head, Division of Crop Protection (up to 14 November 2002), Acting Director (1 June–16 August 2002) and Project Coordinator (Joined on 15 November 2002)

Mr. 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. R. Suseela Bhai, Senior Scientist (Plant Pathology)

Dr. A. Ishwara Bhat, Senior Scientist (Plant Pathology)

Dr. R. Ramakrishnan Nair, Senior Scientist (Genetics and Cytogenetics)

Mr. K. M. Abdulla Koya, Scientist (Selection Grade) (Agricultural Entomology)

Mr. Santhosh J. Eapen, Scientist (Selection Grade) (Nematology)

Ms. N. K. Leela, Scientist (Selection Grade) (Organic Chemistry)

Dr. K. P. M. Dhamayanthi, Scientist (Senior Scale) (Genetics and Cytogenetics) (Transferred on 29 November 2002)

Dr. P. Rajeev, Scientist (Senior Scale) (Agricultural Extension)

Dr. C. K. Thankamani, Scientist (Senior Scale) (Agronomy)

Dr. K. Kandiannan, Scientist (Senior Scale) (Agronomy)

Dr. K. S. Krishnamurthy, Scientist (Senior Scale) (Plant Physiology)

Dr. S. S. Veena, Scientist (Senior Scale) (Plant Pathology) (Transferred on 14 January 2003)

Dr. A. Kumar, Scientist (Senior Scale) (Plant Pathology)

Dr. V. Srinivasan, Scientist (Senior Scale) (Soil Science)

Dr. K. N. Shiva, Scientist (Horticulture)

Technical

Dr. Johny A. Kallupurackal, Technical Information Officer (T-7)

Mr. P. Azgar Sheriff, Technical Officer (T-6)

Dr. P. Hamza Srambikkal, Technical Officer (T-6)

Ms. Utpala Parthasarathy, Technical Officer (T-6) (Joined on 4 December 2002)

Mr. M. M. Augusthy, Technical Officer (T-5)

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

Mr. K. V. Saji, Scientist (Senior Scale) (Economic Botany)

Mr. P. Heartwin Amaladhas, Scientist (Agricultural Engineering) (Transferred on 20 May 2002)

Technical

Mr. V. K. Abubacker Koya, Farm Superintendent (T-7)

Krishi Vigyan Kendra

Dr. T. K. Jacob, Senior Scientist (Agricultural Entomology) and Training Organizer (Joined on 10 December 2002)

Mr. P. S. Manoj, Technical Officer (T-7) (Horticulture)

Dr. S. Shanmugavel, Technical Officer (T-7) (Veterinary Science)

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, Scientist (Senior Scale) (Plant Physiology)

Mr. D. Prasath, Scientist (Horticulture) (Study leave from 31 December 2002)

Administrative

Ms. Enid Savitha, Assistant Administrative Officer

Weather

Weather data for 2002 (Peruvannamuzhi)

Month	No. of rainy days	Rainfall (mm)	Sunshine hours
January	2	16.0	6.8
February	5	90.0	6.7
March	1	3.0	6.7
April	14	204.0	6.8
May	18	570.0	4.8
June	25	807.0	1.3
July	30	580.0	1.7
August	26	728.0	1.7
September	8	120.8	5.4
October	19	556.1	3.3
November	11	232.1	5.6
December	1	26.4	6.7
Total	160	3933.4	57.6

Weather data for 2002 (Appangala)

Month	Temperature (°C)		No. of rainy days	Rainfall (mm)	Humidity (%)
	Max.	Min.			
January	28.4	15.1	0	0.0	64.5
February	30.7	13.9	3	7.8	65.0
March	32.5	16.6	0	0.0	58.7
April	31.1	20.0	5	52.5	73.2
May	30.7	20.0	3	110.5	80.4
June	27.1	19.2	16	303.5	91.9
July	24.6	18.8	31	530.9	95.3
August	24.6	18.6	21	572.1	94.5
September	27.3	17.7	12	185.7	85.8
October	27.3	17.6	14	418.0	84.3
November	28.7	16.6	0	0.0	75.6
December	28.7	10.9	0	0.0	62.7
Total	-	-	105	2181.0	-

सारांश

भारतीय मसाला फसल अनुसंधान संस्थान, कालिकट सवा शताब्दी से अधिक काल से मसालों केलिए एक मुख्य अनुसंधान संस्थान के रूप में सेवा करती आ रही है। इस रिपोर्ट काल में अंकित x वीं पंचवर्षीय योजना काल की शुरूआत में योजना के अन्तर्गत 75% अधिक तथा गैर योजना के अन्तर्गत 30% अधिक अस्थाई बजट आकलन अंकित किया जाता है। इस संस्थान ने स्टाफों केलिए एक कटु मानव संसाधन विकास कार्यक्रम, अनुसंधान कार्यक्रम केलिए एक बहु शैक्षणिक उद्‌गम, अन्तर संस्थानीय सहयोग और प्रभाग केंद्रित के बदले समस्या प्रधान उद्‌गम आदि केलिए कदम उठायी है। संस्थान के प्रमुख अनुसंधान कार्य एवं अन्य प्रमुख कार्य का सारांश यहाँ दिया जाता है।

जननिक संसाधन

केरल और अन्डमान द्वीप समूह से *पाइपर* स्पीसीस की 93 अक्सशनें और काली मिर्च के कृष्ट प्रकारों को संचित किया गया। IPGRI विवरण के आधार पर 110 अक्सशनों को चरित्रांकित एवं सूचीबद्ध किया गया। इलायची की 26 अक्सशनों और संबन्धित वंश के 11 अक्सशनों को जर्मप्लासम में जोड दिया। IPGRI विवरण के आधार पर बहत्तर अक्सशनों की उपजता एवं वानस्पतिक स्वभाव का मूल्यांकन किया गया। चौबीस *जिंजिबर* अक्सशनें और 20 कुरकुमा को जर्मप्लासम में जोड दिया। *गार्सीनिया* स्पीसीस के सात संकलन, *सिनमोमम सल्फोरेटम* का एक संकलन तथा *मिरिस्टिका* के छः वन्य स्पीसीस आदि को जर्मप्लासम में जोड दिया। काली मिर्च की बीस अक्सशनें, हल्दी की 18 अक्सशनें, अदरक की 15 अक्सशनें तथा वैनिला की 100 अक्सशनें आदि को *इन विट्रो* जीन बैंक में जोड दिया।

जर्मप्लासम का चरित्रांकन

काली मिर्च के 14 प्रमुख कृष्ट प्रकारों एवं 10 विमोचित किस्मों के RAPD प्रोफाइल से यह सूचित करता है कि उनमें *फाइटोफथोरा* सह्य किस्में अपना ही एक गुच्छ बनाता है। रूप वैज्ञानिक और कोशिका वैज्ञानिक अध्ययन यह सूचित करता है कि सूक्ष्म प्रवर्धित एवं पारस्परिक रूप से प्रवर्धित काली मिर्च पौधों में कोई भेद नहीं दिखाई पडता है। एलट्टरिया की 13 स्पीसीस और 24 आशाजनक जीनरूप के RAPD प्रोफाइल यह प्रकट करता है कि उन में बहुरूपात्मकता बहुत कम है। RAPD द्वारा अदरक में सूक्ष्मप्रवर्धित सोमाक्लोन की जननिक निष्ठा का विश्लेषण करने पर उनमें विविधता सूचित करता है। हल्दी की सूक्ष्म प्रवर्धित पौधों के RAPD रूपरेखा में प्रोफाइल अन्तर दिखाई पडता है,जबकि माइक्रोरहिजोम से व्युत्पन्न पौधों के प्रोफाइलों में एकता अधिक दिखाई पडता है। RAPD प्रोफाइलों के आधार पर *सी.लौंगा, सी. अरोमटिका, सी. जेडोरिया, सी. मलबारिका* और *सी. अमेडा* जैसे *कुरकुमा* स्पीसीस का अन्तर समझ लिया जा सकता है। हल्दी के 16 अक्सशनों के क्रोमसोम संख्या का

विश्लेषण करने पर उनमें 2n=61से 2n=80 तक का अंतर देखा जाता है।

फसल सुधार

काली मिर्च संकरज (HP-34, HP-105, HP-780, HP-813 और HP-1411) और ग्र प्रजाति (OPKm) पेरुवन्नामूषी (केरल) और वाल्पारै में लगातार अच्छी निष्पत्ती प्रस्तुत करती है। संकलन 1041 और OPKm केरल के चार उत्तर जिलाओं में किसानों के खेत में अच्छी निष्पत्ती प्रस्तुत करती है। अदरक की दो उच्च उपजवाली, उच्च गुणवत्तावाली प्रजाति जैसे "आई आई एस आर महिमा" और "आई आई एस आर रजता" तथा जायफल का एक उच्च उपजवाली, उच्च गुणवत्तावाली क्लॉन चयन, "आई आई एस आर विश्वश्री" को केरल में खेती करने केलिए विमोचित किया गया।

काली मिर्च के दो मानचित्रों को, एक पन्नियूर-1x शुभकरा (करिमुंडा) और दूसरा पी-24 (फाइटोफथोरा सह्य) x शुभकरा को अणुओं के मानचित्र कीतैयारी केलिए विकसित किया गया। पाईपर स्पीसीस के 12 अक्सशनों और काली मिर्च कृष्ट के 8 अक्सशनों से पैतृक बहु आकारिकी निर्धारण करने केलिए DNA वियुक्त किया। काली मिर्च पौधों के पत्तों से RNA वियुक्त करके पाईपरिन जैव संश्लेषण में होनेवाले जीनस तथा प्राप्त अन्य गुणों की पहचान केलिए RT-PCR विभिन्नता दिखाया गया।

इलायची में निष्पादन, संकर ओज और विशिष्ट संयोजन गुण के आधार पर उपजता और पर्ण अंगमारी को सह्यता केलिए आशाजनक 15 संकरज संयोजनों की पहचान की गयी।

मिरिस्टिका मलबारिका पर जायफल का मुकुलन सफल हो गया, मगर सफलता का प्रतिशत कम (30%) होता है। *गार्सीनिया गम्मि गट्टा* को *जी.होमब्रोनियाना* और *जी कोवा* पर मृदुकाष्ठ कलम बाँध दिया जा सकता है जिसके द्वारा 90% सफलता प्राप्त होता है। *जी. इंडिका* में भी *जी. गम्मिगट्टा* पर कलम बाँधने पर 54.5% सफलता अर्जित कर सकी।

गुणवत्ता मूल्यांकन

कृष्ट वन्य पाईपर जर्मप्लासम की गुणवत्ता विश्लेषण किया गया और अक्सशन 5411 में 31.8% ओलिओर्रासिन और 6.2% पाइपरिन अंकित किया जिसके बाद आता है अक्सशन 5442 जिसमें 21.6% ओलिओरसिन और 6.0% पाइपरिन होता है। काली मिर्च को *पी नाइग्रम* और *पी. कोलुब्रिनम* पर विभिन्न तरीके से कलम बांधने पर भी गुण में कोई विभिन्नता नहीं दिखाई पडती है और सभी नमूने, जिसमें, निर्यंत्रित भी शामिल है, पर औसत 3.2% से 4.0% तेल, 3.8% से 4.1% पाइपरिन और 9.5% से 11% ओलिओर्रासिन प्राप्त होते है।

गुणवत्ता मूल्यांकन किये 150 इलायची अक्सशनों में अक्सशन 60, 63, 75 और 273 में 8.0% से अधिक बाष्पशील तेल होता है। अक्सशन 257, 258, 259, 277 और 325 में लगभग 30%α टरपिनिल असिटेट और लगभग 25% 1, 8 सिनोल होता है। गुणवत्ता मूल्यांकन किये 60 अदरक अक्सशनों में गुरुबदनी, कोषिकालन और अक्सशन 121, 260, 340 और 342 में 5.5% से अधिक ओलिओर्रासिन होता है; कोषिकालन और गुरुबदनी में 4% कडा रेशा होता है।

हल्दी में कुरकुमिन अंश के उतार चढाव में स्थान प्रभाव का अध्ययन करने पर यह सूचित होता है कि

कालिकट (केरल) की अपेक्षा नियार्मति (करनाटक) में 20% कुरकुमिन अंश का उतार होता है। कुरकुमिन के जैवसंश्लेषण की प्रारंभिक दशा में PAL की मुख्य भूमिका की पुष्टि करते हुए हल्दी में राइसोम विकास की प्रारंभिक दशा में फिनाइलालनिन अमोनिया लाइस (PAL) को कुरकुमिन के साथ सक्रिय होते दिखाई पडा।

गुणवत्ता मूल्यांकन किये 30 जायफल अक्सशनों में जायफल में सुगंधित तेल के अंश में 7.67% से 13.89% तक तथा जावित्री में 7.48% से 20.99% तक का अंतर देख लेता है। फल और जावित्री दोनों में उच्चतम तेल A9-49 अंकित किया। जायफल में मिरिस्टिसिन के अंश में 2.26% (A11-25) से 29.60% (A11-21) तथा जावित्री में 1.70% (A11-25) से 28.68% (A9-4-11) का अंतर होता है। जायफल में एलिमाईसिन के अंश में 1.51% (A9-37) से 29.71% (A11-26) तक और जावित्री में 1.05% (A9-49) से 29.85% (A9-4-11) तक अंतर होता है।

पप्रिका अक्सशनों के रंग मूल्य में क्रमश:देशज और विदेशी जर्मप्लासम संकलन के लिए 42 से 171 ASTA एकक और 33 से 176 ASTA एकक अंतर होता है।

पादप पोषण प्रबंधन

बुश पेप्पर में वर्मिकम्पोस्ट 1.25 कि ग्राम/ पॉट के दर में लगाने पर मिट्टी में फॉसफोरस की मात्रा में 2.1 से 55.0 मि ग्राम/ कि ग्राम तथा पोटेशियम 103 से 262 मि ग्राम / कि ग्राम तक बढता है और रासायनिक उर्वरक लगाने की अपेक्षा उपजता में 51% से अधिक वृद्धि भी होती है। वर्मीकम्पोस्ट और पर्णकम्पोस्ट उपचार करने पर रिसोस्फियर में रोगाणुक जैवमात्रा बहुत अधिक होता है।

अदरक एवं हल्दी में नाइट्रेट अपचायक और एसिड फॉसफाटेस क्षमता पर सिंक के विभिन्न स्तरों के प्रभाव पर अध्ययन करने पर यह सूचित होता है कि इन एनजाइमों की क्षमता में सिंक स्तर का कोई प्रभाव नहीं होता है। फिर भी मृदा पर लगाने की अपेक्षा पत्तों पर लगाने पर इनकी क्षमता अधिक होती है।

सूखापन का प्रबंधन

जल की कमीवाले जगहों में काली मिर्च संकरजों पर सूखा सह्यता की छान बीन करने पर HP 29 में अधिक जल, कम झिल्ली (Membrane) नाश और SOD क्षमता में अधिकता बनाये रखते देख लेता है। लेकिन जल लगाने पर सभी अक्सशनों में एक जैसा प्रोटीन बैंडिंग पैटर्न दिखाई पडता है और यह नियंत्रण के समान हो जाता है।

सुखा सह्यता के लिए छः इलायची जीन रूप की परीक्षा की और उसमें APG-18 जल लगाने की हालत में उच्चतम होता है। अठाइंस जीनरूप (genotype) में संबंधित जलांश, विशिष्ट पर्ण वजन और रंध्री गणना की परीक्षा करने पर उनमें महत्वपूर्ण विभिन्नता प्रकट हुई। इलायची पर आधारित फसलन रीति में मृदा और जल परिरक्षण के विभिन्न उपायों का मूल्यांकन करने पर सह सूचित होता है कि इलायची के बीच जीवन्त घेरा के रूप में अनन्नास का रोपण करना आशाजनक हो रहा है।

रोग प्रबंधन

फाइटोफथोरा खुर गलन:

46 *फाइटोफथोरा काप्सीसी* वियुक्तियों के रूपवैज्ञानिक

और रोगजनक विभिन्नता पर अध्ययन करने पर उनकी रोगजनकता में महतवपूर्ण भेद सूचित करता है तथा पांच वियुक्तियाँ 98-3, 98-59, 98-146, 98-173 और 02-51 कम विषेली दिखाई पडी।

ग्रीन हाउस में *पी काप्सीसी* के प्रति परीक्षण किये 70 संकरजों में सात अक्सशनों (HP-9, HP-117, HP-477, HP-528, HP-561, HP-599 और HP-1660) को प्रतिक्रिया सह्य दिखाई पडा। 25 कृष्ट प्रकारों में दूसरी बार परीक्षण करने पर 3 अक्सशनों (C-888, C-1199 और C-1204) को प्रतिक्रिया सह्य दिखाइ पडा। सेलह काट्टनाडन को उनकी सह्यता पुष्टि की परीक्षा की गयी और उनमें नौ चयनें (अक्सशन 2420, 2425, 2426, 2428, 2432, 2433, 2466, 2535 और 2575) सह्यतायुक्त देखे गये।

पी कोलुब्रिनम (फाइटोफथोरा काप्सीसी के प्रतिरोधी) के मूलकांड (rootstock) पर कालो मिर्च के सी.वी कर्रिमुडा को कलम बांधने पर किसानों के खेत में उसका अच्छा फल दिखाई पडा और पांचवें साल अधिकतम उपजता 1.062 कि ग्राम / बेल (सूखे) के साथ खुर गलन रोग का प्रभाव रहित भी अंकित किया गया।

इन विट्रो अध्ययन में *पी काप्सीसी* के प्रति कवग और जीवाणुक वियुक्तियों (दोनों का 14) का मूल्यांकन करने पर तीन कवग (IISRF-559, IISRF-563, और IISRF-567) और तीन जीवाणुक वियुक्तियों (IISR-526, IISR-632 और IISR-655) में पी काप्सीसी की वृद्धि निरोध >50% प्रकट हुआ। आशाजनक जीवाणुक वियुक्तियाँ भी ग्रीन हाउस को हालत में रोग का दमन कर सकता है। खेत में लगाने केलिए PGPR गुणन केलिए मौलेसस (0.5%) उचित होता है। जैवनियंत्रण एजेंट जैसे ट्राइकोडरमा हरजियानम और प्स्यूडोमोनस फ्लूरिसन्स केलिए वाहक माध्यम के रूप में अपघटित नारियल जटा कम्पोस्ट का प्रयोग करना उत्तम होता है।

अवरुद्ध रोग

कालिकट और वयनाडु जिलाओं से संकलित अवरुद्ध रोग बाधित पर्ण नमूने के संयोग से रोग लक्षण विज्ञान, रोगवाहक संचारण, इलक्ट्रोन सूक्ष्मदर्शी और सीरम विज्ञान आदि के आधार पर badnavirus को स्थापित किया। इस वाइरस को रोगबाधित पौधों से स्वस्थ काली मिर्च पौधों में कलम बाँधने की तरीके तथा धारित मीली बग (Ferrisia virgata) द्वारा संचारित करता है। कुकुम्बर मोसाइक वाइरस बाधित कालीमिर्च को *निकोटियाना बनतामियाना* और *एन. ग्लूटिनोसा* पर रस निवेशन द्वारा बहुत आसानी से संचारित किया। तम्बाकू से कुकुम्बर मोसाइक वाइरस के निर्मलीकरण केलिए एक प्रोटोकॉल मानकीकृत किया।

स्पाइक झाडना

पन्नियूर 1 के स्पाइकों में द्विलिंगी फूलों के उत्पादन केलिए किये गये निरीक्षण से यह प्रकट हुआ कि अगस्त के समय द्विलिंगी फूलों का प्रतिशत बहुत कम (3.9%) होता है, जो इस किस्म में, विशेषकर उच्च तुंगता में, छितरे हुए को व्यवस्थित करने और स्पाइक झाडने केलिए एक कारण हो जाएगा। पन्नियूर 1 अधिक सुप्रभाव्य होता है और पन्नियूर ५, शुभकरा, पंचमी, बालनकोट्टा और कोट्टनाटन ऐन्थ्रैक्नोज की स्वाभाविक बाधा के सह्य हो गये।

प्रकन्द गलन

प्रकन्द गलन रोग का नैमित्तिक जीव, पित्तियम स्पीसीस

के प्रति दो सौ पांच जर्मप्लासम अक्सशनों का छानबीन किया गया। उनमें तैंतीस अक्सशनें, जो प्रार्थमिक छानबीन में रोग बाधा से बच रहे, दूसरी बार छानबीन करने पर पांच अकसशनें (अक्सशन 6, 17, 130, 155 और 208) आशाजनक हो गये।

जीवाणुक म्लानी

पादप रोगजनकों के समाहरण में छः नये *रालस्टोनिया सोलानसीयरम* संकलनों को जोड दिया अदरक में घ्कड संग्रहों के विश्लेषण वियुक्तियों (28) को 0.70 का गुणांक समता युक्त चार प्रमुख haplotypes गुच्छ बनाया जिसमें दो गुच्छों में जीवाणुक म्लानी का प्रभाव देखता है। मृदा से डी.एन.ए.आर सोलानसीयरम की वियुक्ति केलिए एक प्रोटोकॉल का सुधार भी किया। जीवाणुक म्लानी के प्रतिरोधक केलिए मृदा निवेशन रीति द्वारा छानबीन किये 250 से अधिक अदरक अक्सशनों में दो महीने बाद सभी अक्सशनों में म्लानी दिखाई पडी। पुनर्जनित कुछा अक्सशनों को अतिरिक्त छानबीन केलिए बनाये रखे।

आर. सोलानसीयरम की तापीय मृत्यु माप 30 मिनुट की अवधि में 45.8 °C के रूप में निश्चित किया। राईजोम को अति उद्भासित करने का समय एवं अवधि अनुकूल बना दिया। राइजोम में ताप बना देने पर तथा राइजोम को ताप में रखने के परिणामस्वरूप अति उद्भासित करने के प्रभाव का अध्ययन किया। अति उद्भासित राईजोम की शीत संग्रहण क्षमता पर अध्ययन करने पर यह सूचित होता है कि राइजोम का अति उद्भासित करने के तुरंत बाद एक महीने संभरण करने पर अंकुरण नहीं होता है।

लघु रोग

फसलोत्तर काल में अदरक में होनेवाले दो रोगकारकों को जैसे सूखे गलन रोग के नैमित्तक कारक के रूप में *माइक्रोफोमिना स्पीसीस* और नेत्र गलन रोग के नैमित्तिक कारक के रूप में *फुसेरियम ऑक्सिस्पोरम* की पहचान की गयी। कोलेट्टोट्राइकम स्पीसीस, फोमा स्पीसीस और पेस्टलोटिओक्सिस स्पीसीस *पेरुवन्नामुषी, वयनाड् और कन्नूर से संकलित पर्ण* छिद्र रोग नमूने से संबंधित है और उनकी रोगजनकता भी प्रमाणित की। कारबनडासिम और मानकोजेब कारबनडासिम (मानकोजेब 63% + कारबनडासिम 12%) का एक संयोजन 50 ppm में माक्रोफोमिना स्पीसीस और फुसेरियम स्पीसीस को प्रतिरोधक पाया गया।

नेमटोड का नियन्त्रण

अदरक के चौबीस अक्सशनों और हल्दी के 59 अक्सशनों को रूट नॉट नेमटोड, *मेलोयिडोगने इनकोग्निटा* के प्रति छानबीन करने पर उनमें अदरक के चार (अकसशन 79, 197, 216 और 219) और हल्दी के चार अक्सशनों को (अक्स 54, 56, 57 और 106) प्रार्थमिक छानबीन में आशाजनक पाया गयै।

रिजोबैक्टीरिया की 291 वियुक्तियों के समाहरणों से पांच वियुक्तियाँ (IISR-522, IISR-528, IISR-658, IISR-853 और IISR-859) द्विनेमटोड नाशक कार्य क्षमतावाले (*राडोफोलस सिमिलिस* और *एम इनकोग्निटा* दोनों का दमन करते हुए) की तरह सूचीबद्ध किया।

आशाजनक कवग (*टी. हरजियानम* और *वर्टिसिलियम क्लामिडोस्पोरियम*) और जीवाणुक वियुक्तियों

(पार्स्टोरिया पेंनिटॉन्स) का खेत मूल्यांकन करने पर यह सूचित करता है कि *वी क्लामिडोस्पोरियम* उपचार किये काली मिर्च बेलों की उपजता अन्य उपचारों की अपेक्षा अधिक होता है। चार आशाजनक कवग वियुक्तियाँ (*वी.क्लामिडोस्पोरियम, टी.हर्जियानम, पासिलोमाइसस लिलासिनस* और *स्कोपुलोरियोप्सिस स्पीसीस*) और रिजोबैक्टीरिया IISR-853 और IISR-859) भी काली मिर्च बेलों के पर्णों का पीलापन कम कर देता है।

कीट और नाशक जीव प्रबन्धन

पोल्लू बीटल (*लौंगिटारसस नाइग्रिपनिस*) के प्रति जर्मप्लासम रक्षागृह में उपलब्ध काली मिर्च अक्सशनों के कृष्ट प्रकारों, संकरजों एवं सोमाक्लोनों का छानबीन करने पर तीन कृष्टों के फलों पर 1%से कम कीटबाधा अंकित की।

कर्नाटक के कोडगु जिला में 13 स्थानों के 39 काली मिर्च बागों में काली मिर्च के मूल पर होनेवाले मीली बग वितरण पर अध्ययन करने केलिए किये गये सर्वेक्षण में 7 स्थानों के 16 बागों में कीट बाधा दिखाई पडी। मूल मीली बग के प्रति माइक्रोबियल रोगजनक और एन्टोमोनोपाथोजनिक नेमटोडों (EPNs) का मूल्यांकन करने पर प्रयोगशाला के जैव आमापन (Bioassays) में कीटों के 32% नाश का कारण EPNS सूचित करता है।

जर्मप्लासम रक्षागृह में उपलब्ध अदरक के 485 अक्सशनों का प्ररोह बेधक (कोनोगीतस पंक्टिफरालिस) के प्रति छानबीन करने पर 8 अक्सशनों में 5% से कम प्ररोहों पर कीट बाधा दिखाई पडी। अदरक पर बाधित कीटों को हटाने केलिए जुलाई से अक्तूबर तक पाक्षिक रूप में नीम का तेल 1% या निम्बिसिडाइन 1% छिडकना आशाजनक होता है।

सिगरट बीटल (*लासियोडर्म सरिकॉम*) द्वारा आनेवाली कीटबाधा को रोकने केलिए संभरण सामग्री के रूप में विभिन्न पादप स्पीसीस के सूखे पत्तों का मूल्यांकन करने पर यह दिखाई पडता है कि पोली प्रोफिलिन संभरणी में *क्लोरोडेनड्रोन इनफोरटुनाटम* के पर्ण धूल में सूखे राइजोम को रखकर संचित करना उत्तम होता है। अदरक के 91 सूखे राइजोम को सिगरट बीटल द्वारा पडे हुए हानि का छान बीन किया और 22 अक्सशनों को कीट बाधा रहित दिखाई पडा।

फसलोत्तर प्रौद्योगिकी

बासिलस स्पीसीस और *प्स्यूडोमोनस* स्पीसीस की दस वियुक्तियों को काली मिर्च बीज से वियुक्त किया गया जो काली मिर्च को सफेद काली मिर्च बनाने केलिए उत्तम होता है। स्वच्छ अदरक के संचयन केलिए विभिन्न संभरणियों का मूल्यांकन करने पर जीरो एनर्जी चेंबर (दो दीवारवाले ईंट से बने तथा दीवारों के बीच रेत भरे हुए बारंबार पानी से भिगोये जगह) साफ अदरक के संचयनकेलिए उत्तम दिखाई पडता है।

प्रौद्योगिकी अंतरण

संस्थान ने मसाले उत्पादन प्रौद्योगिकी पर खेत विस्तार कार्यकर्ताओं और शोध कर्मियों केलिए चार प्रशिक्षण कार्यक्रम आयोजित किया। जैव सूचनाओं पर दो *ह्रस्व कालीन प्रशिक्षण कार्यक्रम तथा बागवानी फसलों* केलिए अणु जैविकी प्रौद्योगिकी पर एक प्रशिक्षण कार्यक्रम भी आयोजित किये गये। संस्थान के विभिन्न स्टाफों को प्रशासनिक नियम, संख्यिकी तरीकाएं तथा कंप्यूटर अप्लिकेशन आदि पर संस्थान में ही

पशिक्षण दिया गया। विभिन्न विश्व विद्यालय के स्नातकोत्तर छात्रों द्वारा 67 ह्रस्व कालीन अनुसंधान परियोजनाएँ चालू की और जिसका निरीक्षण यहाँ के वैज्ञानिकों ने किया। संस्थान ने विभिन्न संस्थानो द्वारा आयोजित 16 विस्तार कार्यक्रमों में भाग लिया। 23 दिसंबर 2002 को किसान दिवस मनाया गया और प्रगामी किसानों को सम्मानित किया गया।

काली मिर्च के 55,000 मूल लगाए कतरन, जायफल का 11,100 ग्राफ्ट, 28,000 बीजपौधे और इलायची के 50 कि. ग्राम बीज कैप्स्यूल आदि का उत्पादन करके किसानों एवं अन्य एजेंसियों को वितरित किया। लगभग 850 कि. ग्राम *ट्इकोडरमा* किसानों एवं अन्य एजेंसियों को बेच दिया। विस्तार साहित्य से संबंधित विवरण क्रमिक रूप से अधुनातन बनाया और इंटरनट पर संस्थान के वेबसाइट में प्रस्तुत किया।

कृषि विज्ञान केंद्र

कृषि विज्ञान केंद्र ने किसानों, बेरोजगार युवाओं, स्त्रियों, स्कूल से छूटे बच्चों तथा विस्तार कार्यकर्ताओं आदि केलिए 78 प्रशिक्षण कार्यक्रम आयोजित किये। कृषि विज्ञान केंद्र ने उच्च उपजवाले ह्रस्वकालीन कसावा, उच्च उपजवाले कोलियस, नारियल जटा से कम्पोस्ट बनाना आदि पर किसानों के खेत में महत्वपूर्ण निर्देशन दिया गया। कृषि विज्ञान केंद्र ने किसानों के लिए 6 किसान मेलाएँ व प्रदर्शन तथा 4 पठन दोरा आयोजित की। पशु चिकित्सालय से परामर्श और उपदेशात्मक एवं घर सेवाएँ दी जाती हैं। कृषि विज्ञान केद्र की मदद से कई बेरोजगार ग्रामीण युवकों ने कृषि नर्सरी, वर्मी कम्पोस्टिंग, फल संसाधन तथा बकरी पालन आदि में स्वरोजगार रूपीकृत किया है।

सूचना सेवाएँ

प्रमुख मसालों का क्षेत्र, उत्पादन, निर्यात और अन्य सांख्यिकी का विवरण प्रदान करनेवाले एक वेब पत्रा www.spicestat.org. नये ढंग से प्रारंभ किया। संस्थान के स्टाफों की भलाई केलिए इन्ट्रानट इनफरमेशन हब, स्पाइसनट पारस्परिक उपभोक्ता से मिले जुले विषय सूची को विकसित करनेलायक एक वेब काम में लाया गया। केंद्रीय रोपण फसल अनुसंधान संस्थान, कासरगोड, राष्ट्रीय अनुसंधान केंद्र पुत्तूर और भारतीय मसाला फसल अनुसंधान संस्थान, कालिकट के पुस्तकालयों का संसाधन एवं सुविधाएँ आपस में बांटने केलिए एक नेटवर्क कार्यक्रम प्रारंभ किया।

अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना

अखिल भारतीय समन्वित मसाला अनुसंधान परियोजना (AICRPS) केंद्रों ने अपने पुडिबारी और दापोली केंद्रों में अदरक, हल्दी, ध्निया, मेथी आदि केलिए जननिक संसाधन और नये जर्मप्लासम संग्रहों को मजबुत बना दिया। मडिगेरी में इलायची के CL-629, CL-681 और CL-730 तथा ओ.पी संतति D-237, CL-730, 8-4, D-11 और 7-24-D11 को आशाजनक दिखाई पडा। P-6, D-237 और CL-746 आदि आशाजनक क्लॉन मडिगेरी में सूखा सह्य होते दिखाई पडा। अदरक, हल्दी, धनिया और जीरा के जर्मप्लासम अक्सशनों से विभिन्न रोगसह्य अक्सशनों को पहचान किया। हल्दी अक्सशनों की गुणवत्ता मूल्यांकन करने पर SG - 685 से अधिक सूखे उपज प्राप्त हुए सूचित करता है। धनिया में, Jco-331 में अधिक तेल (0.45%) होता है।

मुडिगेरी में इलायची के कैप्स्यूल उपजता में सूक्ष्मपोषक जैसे बोरोन, और मोलिब्डिनम का प्रभाव होता है। अदरक एवं हल्दी उत्पादन के लिए चितापल्ली में एक पैकेज पद्धति मानकीकृत किया। येरकाड में एक उर्वरक पैकेज, जिसमें जैव उर्वरक लगाना भी शामिल होता है, लौंग और जायफल केलिए मानकीकृत किया। Zn, Fe, Mn और Cu लगाने पर धनिया और कलौंजी की उपजता एवं गुणवत्ता में वृद्धि दिखाई पडी। जीरा और मेथी की बुआई केलिए अनुकूलतम काल निश्चित किया गया।

पन्नियूर में काली मिर्च केलिए प्रति दिन दो लिटर पानी सींचने से स्पाइक की संख्या, हरे फल की उपजता और स्पाइक की लंबाई में वृद्धि पा सकी। धोली में अदरक केलिए सूक्ष्मपोषक Fe को 1% के दर में पत्तों पर छिडकने से उपजता में महत्वपूर्ण वृद्धि दिखाई पडी। जायफल के कायिक प्रवर्धन में एपिकोटिल ग्राफ्टिंग की अपेक्षा अप्रोच ग्राफिटंग (90%) में सफलता अधिक दिखाई पडी।

काली मिर्च की *फाइटोफ्थोरा* खुर गलन के नियंत्रण करने केलिए Metalaxyl Gold MZ और *ट्राइकोडरमा* स्पीसीस का बहुत प्रभाव देख लिया। केरल के इडुक्की जिला के उच्च तलों में काली मिर्च पर होनेवाले मसल शल्क (लेपिडोसफस पाइपरिस) के नियंत्रण करने केलिए फल तोडने के बाद पाक्षिक अन्तराल में मोनाक्रोटोफॉस (0.05%) या डायमेतोयट (0.05%) दो बार छिडकना प्रभावी होता है।

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