Plant Biotechnology Research at the Kerala Agricultural University

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FOREWORD

The use of biotechnology to modify plants represents a significant advance in plant science. It facilitates creation of new genetic variability and can complement the ordinary breeding strategies. Biotechnology offers opportunities to increase the availability and variety of food, increasing overall agricultural productivity, while reducing seasonal variations in food supplies. Through the introduction of pest-resistant and stress-tolerant crops, biotechnology could lower the risk of crop failure under difficult biological and climatic conditions. Bio-availability of nutrients and the nutritional quality of products are ensured. Biotechnology can speed up conventional breeding programmes and provide farmers with disease-free planting materials. It can also provide diagnostic tools for various plant diseases. Biotechnology should form part of an integrated and comprehensive agricultural research and development programme in the public sector, that gives priority to the problems of the poor. Capacity building for biotechnology research in public sector is very much relevant. In India, a strong scientific base in agricultural biotechnology research is being built up and strengthened, equipping it with infrastructure, trained human resources, information and communication.

Biotechnology research at the Kerala Agricultural University has been in progress for the last two decades. More than thirty scientists and hundred post graduate students have been involved. One hundred and eighty research projects, including thirty six externally aided projects were in operation. Facilities for research have been established at the College of Horticulture, Vellanikkara, College of Agriculture, Vellayani and a few research stations. Several scientists have been trained in national and international institutions. Several of them could bag prestigious national awards. The Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara was awarded the "Biotech Product & Process Development & Commercialisation Award 2003" of the Department of Biotechnology, Ministry of Science and Technology, Government of India. M.Sc. (Ag.) Plant Biotechnology programme was initiated in 2003. Training programmes, are regularly offered to the unemployed youth, officers of the Department of Agriculture, and various development agencies, teachers etc. Transfer of Technology to farmers on management of tissue culture plants has been effectively done. Several protocols could be commercialized. Also, the University is providing R&D support and consultancy to several biotechnology programmes of the developmental agencies of the state.

The activities of the Kerala Agricultural University in plant Biotechnology for the last two decades has been documented in this compendium. It places on record the efforts, perseverance and commitment of all those concerned. I am happy to offer my felicitations to the authors of this valuable document.

Kerala Agricultural University
Vellanikkara, Thrissur
1 January 2005

Prof. (Dr.) K. V. PETER
Vice-Chancellor

PREFACE

Scientific advances in agriculture provide the best opportunity to meet the demands of the growing world population. Use of new technologies, such as biotechnology can help overcome many of the constraints of the conventional agriculture. Biotechnology should form part of an integrated and comprehensive agricultural research and development programme that gives priority to the problems of the poor. A greater dependence on biotechnology is quite obvious. Over the past decade, application of biotechnology to the problems of agriculture has yielded significant productivity gains.

Strong and dynamic capacity is the most important requisite for successful and sustainable application of biotechnology in agriculture. The Kerala Agricultural University has initiated biotechnology programmes in the early eighties itself. Facilities have been established at the College of Horticulture, Vellanikkara, College of Agriculture, Vellayani and a few research stations. Several faculty members have been trained in national and international institutions. About thirty scientists and one hundred post-graduate students have been involved in one hundred and eighty research projects. Thirty six externally aided projects have been implemented.

The efforts of the scientists and students could yield fruitful results. Several of them could bag prestigious national awards. The Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara was honoured with the "Biotech Product & Process Development & Commercialisation Award 2003" of the Department of Biotechnology, Ministry of Science and Technology, Government of India. Teaching at undergraduate and post-graduate levels, training programmes, transfer of technology and extension programmes, consultancy etc constitute the other areas of activities. The Biotechnology Project Coordination Group has been providing effective guidance to all these efforts. This book is an attempt to document such activities. The various technologies, products and processes developed at the biotechnology centers of the University have been summarized in this compendium.

We are thankful to all concerned in making it possible to bring out this useful compendium of biotechnology research and development activities at KAU. The University Press has done commendable work to bring out this publication in time.

AUTHORS

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PLANT BIOTECHNOLOGY RESEARCH AT THE KERALA AGRICULTURAL UNIVERSITY

INTRODUCTION

Plant Biotechnology research has been initiated at the Kerala Agricultural University in 1981. The initial emphasis was on *in vitro* propagation of horticultural crops. Protocols were developed for rapid *in vitro* cloning of elite plants of jack, banana, pineapple, black pepper, cardamom, ginger, turmeric, vanilla, cinnamon, orchids, anthurium, rose, gladiolus, foliage plants, medicinal plants (*Kaempferia galanga, Plumbago rosea, Aristalochia indica., Smilax* sp., *Trichopus zeylanicus, Tylophora indica, Coscinium fenestratum, Bacopa monnieri, Centella asiatica, Aegle marmelose, Gymnema sylvestre, Rubia cardifolia, Trichosanthes cucumerina, Createaeva magna, Stevia rebaudiana, Spathyphyllum cannefolium, Mycrophyllum aquaticum* and *Holostemma annulare*) and forest species (*Dalbergia latifolia, Ailanthes triphysa, Pterocarpus marsupium* and *Tectona grandis*). The tissue culture plants were evaluated for their field performance in different agro-climatic conditions. Research on the *in vitro* propagation of mango, cashew, malabar tamarind, ornamental plants and medicinal plants is in progress.

Subsequently, research on crop improvement using tissue culture techniques was taken up. Seed set in ginger could be achieved for the first time using *in vitro* pollination and fertilization. Inter-specific hybridisation in okra, as a prelude for imparting disease resistance, was successful using embryo rescue techniques. Research on evolving disease resistant plants *via in vitro* techniques in pepper, ginger and rice is in progress. Thirty somaclonal variants of pepper that showed field tolerance to *Phytophthora* foot rot disease, are being evaluated further.

One hundred and forty six research projects were taken up in biotechnology, so far (Annexure I). Thirty six research projects were funded by agencies like Department of Biotechnology, Indian Council of Agricultural Research, BARC, Kerala State Council for Science, Technology and Environment, Department of Agriculture and US-India Funds (Annexure II). The external funding for the various externally aided research projects in biotechnology, so far, is to the tune of Rs. 6.5 crores. The funding was helpful in establishing biotechnology centers at the College of Horticulture, Vellanikkara and College of Agriculture, Vellayani, and Plant tissue culture laboratories at the Regional Agricultural Research Stations at Ambalavayal, Kayamkulam, Pattambi and Kumarakom and at the College of Agriculture, Padannakkad.

Thirty seven scientists (Annexure III) are associated with biotechnology research. Fifteen scientists had training in reputed national and international institutions in frontier areas of plant biotechnology and molecular biology.

1. RESEARCH HIGHLIGHTS

Fruits

Protocol for *in vitro* propagation of jack *via* enhanced release of axillary buds was evolved. The *ex vitro* establishment problem of jack plantlets was studied in detail and protocol evolved for successful field establishment.

Protocols for *in vitro* propagation of banana varieties Nendran and Red Banana were evolved and commercialized. Tissue culture Nendran banana was found suitable for high density planting in uplands for better returns. During the early stages of growth, the vegetative characters recorded higher values for the sucker derived plants whereas during the later periods, the plant height, girth, number of functional leaves per plant and the total number of leaves were more in tissue culture plants. The superiority of tissue culture plants was evident in the 43 per cent increase in yield. Application of higher dose of fertilizers (NPK 300:115:450 g/plant) recorded 9.0 per cent increase in yield over the recommended dose of 190:115:300 g NPK / plant.

Protocol for *in vitro* propagation of pineapple was evolved. The plantlets were evaluated for their field performance.

Progress could be made in the *in vitro* propagation of mono and polyem bryonic mango varieties, *via* somatic embryogenesis from nucellar tissue.

RAPD analysis of the clones and intraclones of banana, papaya and mango has been done.

Spices

Protocols for *in vitro* propagation of pepper varieties were evolved. Large scale multiplication of high yielding varieties of black pepper was undertaken and subjected to detailed field evaluation.

Plants showing tolerance to *Phytophthora* foot rot disease could be evolved from unscreened callus *in vitro*, revealing the possibility of exploiting somaclonal variation for diseases and other stress tolerance.

Based on isoenzyme variation, nine species of *Piper* could be grouped into three groups.

β1-3 glucanase activity in infected *"Piper nigrum"* was found increased by 50 per cent within four days after inoculation with the pathogenic fungi while no significant variation for enzyme activity was observed in *"Piper colubrinum"*, the species reported to be resistant to the disease, within the same period of time. However, the endogenous enzyme activity measured in *"P. colubrinum"* was even more than the elevated range in *"P. nigrum"* indicating its role in the resistance mechanism.

Using RAPD analysis, 44 varieties of *Piper nigrum* were grouped into basically 11 groups with NE collection forming a group totally separate from all others. AFLP analysis was also carried out to group the pepper varieties. Characterization of piper species was done through RAPD and AFLP assay.

Methods could be standardized for *Agrobacterium tumefaciens* mediated genetic transformation in pepper.

DNA sequences encoding three defense proteins *viz.* ß-1,3-glucanase, hydroxyl methyl glutaryl CoA reductase (hmgr) and chitinase have been cloned from different crop species as well as wild plants. Three sequences have been deposited in databank and others are under processing.

Protocol for *in vitro* propagation of ginger was evolved. The plantlets were evaluated for their field performance.

Methods were standardised for *in vitro* pollination and fertilisation in ginger. Out of the several seeds produced by this method, one seed could be made to germinate. This is the first report on seed set in ginger.

Methods could be standardized for *Agrobacterium tumefaciens* mediated genetic transformation in pepper.

Isoenzyme banding pattern of 39 genotypes of *Curcuma longa* was studied for esterase, peroxidase and GOT. The similarity index among the genotypes ranged from 0.39 to 1.0. The genotypes were classified into 21 groups with similarity index 1.0 among the members. Eighteen species of *Curcuma* were also grouped based on isoenzyme similarity.

Protocols for *in vitro* propagation of cardamom was evolved. The plantlets were evaluated for their field performance.

Progress could be made in the *in vitro* propagation of clove *via* enhanced release of axillary buds.

In vitro propagation of cinnamon *via* enhanced release of axillary buds could be standardized.

Methods could be standardized for *Agrobacterium tumefaciens* mediated genetic transformation in bell pepper.

Cashew

Remarkable progress in the *in vitro* propagation of cashew *via* enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis was achieved. RAPD analysis of the varities of cashew has been done.

Cocoa

Progress in the *in vitro* propagation of cocoa *via* enhanced release of axillary buds was achieved.

Ornamental plants

In vitro propagation of the orchid, *Dendrobium* variety Sonia 17 was standardized and commercialized. Methods could be standardized for the *in vitro*

propagation of *Phalaenopsis*. Methods could be standardized for *Agrobacterium tumefaciens* mediated genetic transformation in the orchid, *Dendrobium* variety Sonia 17. RAPD analysis of the varities and hybrids of *Dendrobium* has been done. *In vitro* mutagenesis of the orchid, *Dendrobium* variety Sonia 17 was standardized and the mutants are being evaluated.

Protocol for *in vitro* propagation of anthurium was standardized and commercialized. Cost effective methods and devices for home scale adoption of *in vitro* propagation in anthurium could be developed.

Protocol for *in vitro* propagation of gladiolus could be evolved. *In vitro* corm production could be induced.

Methods for *in vitro* propagation of rose, mussaenda and bougainvillea could be standardized.

In vitro mutagenesis in tube rose was standardized and the mutants are being evaluated.

Medicinal plants

Protocols were developed for the mass *in vitro* clonal propagation of *Kaempferia galanga* and for the field establishment of the plantlets. The performance of the tissue culture derived plants were comparable to the conventional plants. Regeneration through organogenesis as well as embryogenesis was obtained.

Protocols for the rapid *in vitro* multiplication of *Holostemma annulare* were standardized. Synthetic seeds could be produced by encapsulating the nodal segments with sodium alginate and complexing with calcium chloride.

Protocol for *in vitro* propagation of *Trichopus zeylanicus* was evolved. Plantlets were successfully transferred to soil.

Protocol for *in vitro* propagation of *Coscinium fenestratum* was developed. Plantlets were successfully transferred to soil.

Protocol for *in vitro* propagation of Gurmor (*Gymnema sylvestre*) was developed. The medium for saponin production from suspension culture was standardized.

Protocol for *in vitro* propagation of *Aristolochia indica* was developed and the plantlets were established in the field.

Protocol for *in vitro* propagation of *Aegle marmelos* was developed.

Protocol for *in vitro* propagation of *Rubia cordifolia* was developed.

Protocol for *in vitro* propagation of *Trichosanthes cucumerina* was developed.

Protocol for *in vitro* propagation of *Crataeva magna* was developed.

Progress in the *in vitro* propagation of Indian gooseberry *via* enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis could be made.

RAPD analysis of the ecotypes of several medicinal plants has been done.

Methods could be standardized for the in vitro production of secondary plant metabolites from *Sida* spp., *Gymnema sylvestre, Coscinium fenestratum* and *Tinospora cordifolia*.

Vegetables

Embryo rescue was successfully done in okra for distant hybridisation, involving *Abelmoschus esculentus* and *A. moschatus*, for evolving plants resistant to yellow vein mosaic disease.

Forest species

Protocols for *in vitro* propagation of *Dalbergia latifolia via* enhanced release of axillary buds as well as for ex *vitro* establishment of plantlets were evolved.

Protocol for *in vitro* propagation of *Pterocarpus marsupium* was developed.

Protocol for *in vitro* propagation of *Crataeva magna* was developed.

Protocol for *in vitro* propagation of *Ailanthus triphysa* was developed.

Protocol for *in vitro* propagation of *Tectona grandis* was developed.

Genetic diversity in natural teak populations and teak provenances of the Western Ghats region were estimated through AFLP technique.

2. *IN VITRO* PROPAGATION

In vitro propagation is by far the most widely exploited area of plant biotechnology, catering to the demands of farmers for quality planting materials. In Kerala, the production and productivity of most of the crops are below satisfactory levels. Poor genetic stock and inferior planting materials are among the major factors responsible for the situation. The scenario can be changed remarkably with the use of quality planting materials. *In vitro* clonal propagation has great relevance in this context. Enhanced release of axillary buds is extensively being used for the clonal propagation of plants. Direct somatic organogenesis and embryogenesis can also be used for the purpose. The summary of work done on the *in vitro* propagation of crop plants, at the Kerala Agricultural University is presented below:

a. FRUITS

Jack

Procedures were standardized for *in vitro* clonal propagation of jack. Plantlets could be produced *via* enhanced release of axillary buds from shoot apices of fresh stem sprouts of jack trees. Murashige and Skoog medium supplemented with GA and activated charcoal was identified as the suitable culture establishment medium, in which the explants were incubated in darkness for four weeks with repeated sub-culturing. The cultures were then exposed to light for two weeks, after which the growing shoot apices were transferred to the proliferation medium (MS + BA + NAA + adenine sulphate + insoluble PVP). Shoots from the proliferation medium were

transferred after five weeks to an elongation medium (MS + BA + NAA + insoluble PVP). The shoots were then cultured on MS medium containing activated charcoal for two weeks. For *in vitro* root induction, the shoots were cultured in darkness in half MS + IBA for six days and then transferred to half MS without plant growth substances for root elongation. Just after the appearance of the roots the plantlets were hardened by exposure to high light intensity for one week. The plantlets were then transferred to sand containing vesicular arbuscular mycorrhizae and subjected to a gradual hardening process before transferring to the garden pots kept in the open field conditions. Stress treatments of the explants (like cold shock and heat shock) improved the *in vitro* response. Cytological examination revealed stability of chromosome number in the plantlets. An efficient protocol for the ex vitro establishment of the plantlets could be evolved after analyzing morphological, histological and physiological peculiarities of the plantlets. Inoculating the potting medium with vesicular arbuscular mycorrhiza favoured 80 –100 per cent survival of the plantlets. Plant growth was significantly increased.

Mango

Techniques could be standardized for the induction, initiation, maturation and germination of somatic embryoids from the nucellar tissue isolated from 30-45 days old fruits of Neelum, Banglora and Vellari Manga. The treatment half strength Murashige and Skoog basal medium containing 2,4-D 5.0 mg/l, GA3 5.0 mg/l, glutamine 400 mg/l, coconut water 200 ml/l, activated charcoal 500 mg/l, sucrose 60 g/l and agar 6.0g/l was the best in induction of embryogenic callus. The treatment 2,4-D 4.0 mg/l + BA 1.0mg/l + GA3 5.0 mg/l in half strength MS basal medium supplemented with glutamine 400 mg/l, CH 500 mg/l, sucrose 60.0 g/l, CW 200 ml/l and agar 6.0 g/l was the best in initiating somatic embryos from the induced nucellus of the three varieties. An attempt was made to induce somatic embryogenesis using cytokinins, without depending on auxin, since the nucellar tissue of mango consists of pre-embryogenic determined cells. In the variety Neelum BA (8.0 and 16.0 mg/l) and kinetin (8.0 to 32.0 mg/l) were as effective as 2,4-D in inducing somatic embryogenesis. This finding is significant in that the deleterious effects of 2,4-D in the later development of somatic embryoids, which was reported in several instances, could be avoided. Better morphology of the somatic embryoids resulted during the initiation as well as the later stages of embryogenesis. The variety Banglora responded better to BA (8.0 and 16.0 mg/l) and kinetin (8.0 mg/l) than to 2,4-D (5.0 mg/l). Better response was observed in the variety Neelum. Instances of direct somatic embryogenesis were observed in Neelum and Banglora. Polyembryonic variety Vellari Manga showed better response to induction treatments than the mono embryonic varieties. This variety responded more to treatment involving BA than to that involving kinetin and 2,4-D. Direct somatic embryogenesis was also more in this variety. The somatic embryoids passed through globular, heart shaped, torpedo and cotyledonary stages. B5 major salts with MS minor salts in combination with 40 g/l sucrose, 10 g/l PVP, 200 ml/l coconut water, 5.0 mg/l ABA, 100 mg/l casein hydrolysate and 6.0 g/l agar were the best suited

for the maturation of the somatic embryos. Major focus of research work during the last two years was on inducing normal germination of somatic embryoids. Treatments involving basal media, plant growth substances, casein hydrolysate, osmotic regulants, ethylene inhibitors, and coconut water were tried for inducing normal germination of the embryoids. The positive influence of cobalt chloride, casein hydrolysate and coconut water on germination of embryoids of both mono and poly embryonic mango varieties could be well established. A basal medium consisting of B5 major salts and MS minor salts supported the highest percentage of germination of somatic embryoids in Neelum and Vellari Manga. However, abnormalities in germination were observed in most of the cases. Normal embryoids had two cotyledons, a hypocotyl, a terminal shoot primordium and a root primordium. In abnormal embryoids one or more of these were either lacking or underdeveloped. In some cases the root pole dominated and the shoot pole remained underdeveloped. Some of the somatic embryoids had a single cotyledon or a rosette of many cotyledons fused together. Excellent germination of somatic embryos was observed in a liquid medium consisting of half strength B5 macro salts and full strength MS micro salts and supplemented with GA3 1.0 mg/l + glutamine 400 mg/l + sucrose 30 g/l. The plantlets formed had normal morphology of roots and shoots. In such instances the embryoids had normal root and shoot poles. The shoot pole emerged rapidly and elongated and produced fully expanded green leaves. These plantlets were similar in appearance to the zygotic seedlings, except for their smaller size.

Banana

The protocol of *in vitro* propagation of Nendran and Red banana could be developed. Shoot apex explants of 2.0 to 2.5 cm, collected from peeper suckers of Red Banana could be effectively surface sterilized using mercuric chloride solution (0.1 per cent) for 15 minutes. The best culture establishment medium was MS supplemented with BA 2.0 mg/l, sucrose 30.0 g/l, agar 6.0 g/l and coconut water 200 ml/l. After three weeks the explants are taken out and cut longitudinally and sub-cultured to a medium of the same composition. After two weeks they are sub-cultured to a shoot proliferation medium (MS medium supplemented with BA 5.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l). The best root inducing medium was identified as MS supplemented with IBA 1.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l. Large number of plantlets could be successfully planted out in the field. The protocol of *in vitro* Nendran banana production was also developed and perfected. Tissue culture Nendran bananas can be planted in higher density planting in uplands for better returns. The recommended spacing is 2m x 3m with two plants per pit, thereby accommodating 3332 plants per hectare. The Cost : Benefit ratio is 1 : 1.67 as against 1 : 1.56 in 2m x 2m spacing. The inter spaces can be utilized for raising crops like amaranths, cowpea and okra during first four months. During early stages of growth, the vegetative characters recorded higher values for the sucker derived plants whereas during the later periods, plant height, girth, number of functional leaves per plant and the total number of leaves were more in tissue culture plants. Total leaf area, leaf area index, leaf area duration,

crop growth rate, net assimilation rate, the time taken for bunch emergence, duration of crop and number of suckers per plant of tissue culture plants showed significant superiority over the sucker progenies. Tissue culture plants showed superiority in dry matter production, nutrient uptake and also fruit quality. The study could prove the superiority of tissue culture plants in which the highest yield of 12.2 kg obtained was 43 per cent more than the lowest yield recorded for sucker derived plants. Application of higher dose of fertilizers (NPK 300:115:450 g/plant) recorded 9.0 per cent increase in yield over the recommended dose of 190:115:300 g NPK / plant.

Pineapple

Reliable protocols could be evolved for the *in vitro* propagation of pineapple. Explants could be successfully disinfected by treating with emisan 0.1 per cent for 35 minutes (shoot tip explants) or 10 minutes (lateral bud explants). Culture establishment and growth initiation were better in MS medium supplemented with BAP 3.0 mg/l. Adventitious bud initiation was maximum in MS medium supplemented with BAP 4 mg/l + NAA 0.5 or 1.0 mg/l. Rate of multiplication was higher in liquid medium under shake culture. Fast shoot regeneration and increased vigour of shoots were resulted in growth regulator free MS medium. *In vitro* rooting was also fast in MS medium without in any growth regulator. For ex *vitro* rooting, dipping the shoots for a period of one hour in IBA 200 mg/l solution was the best. Among the potting media tried, Soilrite was found most ideal for ex *vitro* establishment and growth of pineapple plantlets. During early stage of crop growth of pineapple cv. Mauritius, sucker derived tissue culture plants recorded higher values for almost all the vegetative characters studied. Total dry matter production and dry matter partitioning were higher for tissue culture plants of elite accessions. They also showed early flowering with longer flowering phase. In the fruit and yield parameters also superiority of tissue culture plants of elite accessions, over that of unselected bulk, was obvious. Among the elite accessions TK 3 (T1) and KT 5(T2) performed well and recorded the maximum estimated yield (77 t/ha), whereas tissue culture plants of unselected bulk recorded a yield of 49 t/ha only. All the tissue culture plants including unselected bulk produced more number of suckers and slips than sucker derived plants. Fertilizer dose of 12:6:12 g/plant NPK applied in six splits at 0, 1, 2, 3, 6 and nine months after planting recorded less crop duration of one month in all tissue culture plants, compared to sucker-derived plants. Tissue culture plants of pineapple hybrids were used for multi location field evaluation of performance. The quality attributes of the fruits of tissue culture plants were comparable to those of the sucker- derived plants.

A detailed survey was conducted at the major pineapple growing areas, namely Vazhakulam and Muvattupuzha region of Ernakulam district (Kerala). Five elite clones of Mauritius variety with higher yield and other desirable fruit charater were selected. Propagules from the selected clones were planted at Vellanikkara and utilised for mass multiplication through refined *in vitro* propagation techniques.

Protocol for large scale multiplication of pineapple var. Kew was also standardized. Shoot tips were collected from healthy young suckers. Of the various media tried for culture establishment, MS supplemented with BA was the best. The established shoot tips were sub-cultured to MS medium with BA and NAA for the production of adventitious buds. The adventitious buds on sub-culturing to MS medium with BA produced multiple shoots. The shoots were rooted in MS basal medium or half MS medium with IBA. The plantlets after subjecting to a process of hardening were transferred to suitable potting medium. In order to reduce the cost of production of *in vitro* plantlets, ex *vitro* rooting with IBA treatment and subsequent hardening of plantlets was standardized for pineapple. About 3000 TC plants were produced and distributed to farmers of Thrissur, Ernakulam and Kollam districts for field evaluation. The yield of TC plants in the field was comparable with that of suckers. However, the fruit size was slightly less in TC plants and the duration was slightly more, compared to sucker derived plants.

b. ORNAMENTALS

Orchids

Protocol could be evolved for the *in vitro* propagation of orchid *Dendrobium* variety Sonia 17. The nodal explants were surface sterilised using mercuric chloride (0.1 per cent) for 10 minutes. The culture establishment medium was identified as Knudson C basal medium + BA1.0 mg/l + NAA 1.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l. Sub-culture to a proliferation medium (half concentration of MS basal medium + BA 2.5 mg/l + IAA 2.0 mg/l + coconut water 150 mg/l + sucrose 30.0 g/l + agar 6.0 g/l resulted in multiple shoot formation. Root induction was observed on half concentration of MS basal medium + kinetin 2.5 mg/l + IBA 1.0 mg/l + coconut water 150 mg/l + sucrose 30.0 g/l + agar 6.0 g/l. Large number of plantlets could be established in pots containing small brick and charcoal pieces or in coconut husks, in a mist chamber.

Basal portion of shoot was found to be the best explant for the *in vitro* propagation of *Phalaenopsis*. Murashige and Skoog medium at 1/4, 1/2, and 3/4 concentration and Vacin and Went medium were identified as the most suitable basal medium. Sucrose 15.0 g/l favoured both multiple shoot formation and protocorm like body (PLB) formation. BA 5.0 mg/l + 2,4-D 2.0 mg/l + NAA 2.0 mg/l favoured both multiple shoot formation and PLB formation from *in vitro* shoots. BA 25.0 mg/l + adenine 10.0 mg/l + NAA 1.0 mg/l favoured PLB formation from *in vitro* leaf and root explants. Peptone 1000 mg/l improved multiple shoot formation. Coconut water 150 to 250 ml/l favoured PLB formation. The highest number of roots per micro shoot was recorded by MS medium at 1/4 concentration supplemented with BA 5.0 mg/l + NAA 2.0 mg/l + 2,4-D 2.0 mg/l + coconut water 150 mg/l and sucrose 30.0 g/l. Coconut husk was the best medium for planting out. Hanging the plants in the orchidarium with high humidity and fortnightly spraying with 17:17:17 complex fertilizer at 0.1 per cent recorded the highest survival rate and growth of plantlets.

Techniques could also be standardized for the *in vitro* propagation of *Aranthera* (Annie Black). Protocorm like bodies were obtained in the embryo culture of three *Dendrobium* types *viz.*, Betty Ho, Amoemum and Uniwy. The protocol for embryo culture for two *Dendrobium* types viz., Madame Pompadour and Kasem White were standardized. Meristem culture was attempted in Kasem White, Betty Ho and a hybrid type. Somatic embryogenesis was attempted in leaf sheath cultures.

Culture media and conditions were standardized for the *in vitro* propagation of *Dendrobium* Sonia and Ceasu Alba x Kasim Gold, *via* embryo culture. Callus raised from leaflets of *Dendrobium* hybrids subjected to varying doses and duration of ethyl methane sulphonate showed significant variation in fresh weight and rate of shoot bud induction.

Hybrid *Dendrobium* plants could be established *via* embryo culture. In vitro clonal multiplication of the promising hybrids was done.

Anthuriums

Five varieties of *Anthurium andreanum*, namely, Dragon's Tongue, Fla King, Pompon Red, Honeymoon Red and Nitta were subjected to micro-propagation studies. Surface sterilization was the most effective with mercuric chloride 0.1 per cent for ten minutes. The callusing response was the highest in the variety Dragon's Tongue (50 per cent) on modified Murashige and Skoog medium containing quarter strength ammonium nitrate and supplemented with BA 0.5 mg/l, 2,4-D 0.5 mg/l, sucrose 30.0 g/l and agar 6.0 g/l. Regeneration from the callus was observed only in the variety Dragon's Tongue on a regeneration medium of MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l. Leaf disc was the best explant. Transfer of the regenerating cultures to an intermediary medium containing activated charcoal 1.0 g/l promoted leaf development and shoot growth. Further sub-culturing to an MS medium supplemented with kinetin 0.5 mg/l + IAA 8.0 mg/l showed the highest increase in the number of shoots, whereas the best response for increase in the number of leaves was obtained with kinetin 0.5 mg/l + IAA 16.0 mg/l. Agar 6.0 g/l was found superior to agarose as the gelling agent. All the treatments tried for shoot proliferation produced satisfactory number of roots and hence a separate rooting phase was not necessary. The rooted plantlets had a high survival rate (60 per cent) when planted out in pots containing sterile sand.

Gladiolus

Techniques could be standardized for the *in vitro* propagation of gladiolus, using corm axillary bud and cormal tip explants. Treatment with 0.2 per cent mercuric chloride for ten minutes was effective for surface sterilization. Culture establishment was observed in MS medium supplemented with BA 1.0 to 4.0 mg/l. Multiple shoot formation was the best on MS medium containing BA 1.0 mg/l and NAA 0.5 mg/l. These when transferred to MS medium devoid of plant growth substances resulted in elongation of shoots. The elongated shoots produced maximum number of roots in

MS medium containing 1.0 mg/l IBA. *In vitro* production of corms could be induced by culturing the shoots in liquid MS medium supplemented with 30.0 g/l sucrose and 0.5 mg/l NAA. Size of the corms ranged from 2.3 to 6.4 mm.

Protocol for the *in vitro* clonal propagation of two promising gladiolus varieties, namely Peach Blossom and Tropic Seas was developed. Kinetin 2 mgl^{-1} + NAA 0.1 mgl^{-1} induced earliest bud initiation in both the varieties. However further survival and shoot growth were better in treatments involving BA (2 and 4 mgl^{-1}) and its combination with auxins. BA 4mgl^{-1} + NAA 0.5 mgl^{-1} resulted in the production of the highest number of shoots. The highest shoot proliferation was obtained with BA 4 mgl^{-1} + NAA 0.5 mgl^{-1} in both the varieties. Sucrose 40 gl^{-1} in the shoot proliferation medium recorded the highest proliferation of healthy and vigorous shoots in both the varieties.

Rose

Techniques were standardized for the *in vitro* propagation of rose. Nodal explants were best established on MS basal medium supplemented with BA 2.0 mg/l and 2,4-D 1.0 mg/l. Shoot proliferation was achieved on MS medium containing BA 2.0 mg/l and GA 1.0 mg/l. The micro cuttings were rooted *in vitro* on half strength MS medium supplemented with IAA 2.0 mg/l. Successful hardening and *ex vitro* establishment of plantlets could be achieved by surface inoculation with germinated spores of vesicular arbuscular mycorrhizae, *Glomus etunicatum*. One reddish yellow mutant could be identified, by subjecting the cultures to gamma irradiation at 30 Gy. Another mutant with increased number of petals was also obtained from 40 Gy treated explants. Treatment with chemical mutagens was not successful, in obtaining a desirable mutant.

Schefflera

Culture was formed from immature and young schefflera leaves and the callus production was good with 2,4-D 1-2 mg/l and NAA 10-12 mg/l but the calli did not respond to caulogenesis. In direct organogenesis, axillary bud break from nodal explants was noticed on MS medium with BA 0.5 mg/l and the shoot growth was the best with BA 5.0 mg/l. The *in vitro* developed shoots were rooted in the medium supplemented with NAA 3.0 mg l^{-1} + IBA 0.3 mg l^{-1}.

c. PLANTATION CROPS AND SPICES

Pepper

In vitro propagation protocols could be evolved for the different varieties of pepper *viz.*, Panniyur-1, Panniyur-2, Panniyur-3, Panniyur-4 and Panniyur-5. Nodal segments were used as the explant. The combination of ethyl alcohol (50 per cent) and mercuric chloride (0.1 per cent) was used as the surface sterilant. Half MS basal medium supplemented with BA served as the culture establishment medium. Callus culture could also be established using leaf segment explants. Callusing was observed within three weeks. Nodal segments also started bud break and sprouted within three weeks. Fungal growth from the surface of the explants, phenolic interference and

systemic bacterial contamination have been observed as the major limiting factors that reduce the culture establishment rate. A nitrogen fixing bacterium, *Beijerenckia indica*, was observed to be present systemically. Two strains of the bacterium were identified. The bacterium was found to be both ecto- and endo-phytic. A combination of IAA and BA in the basal medium favoured multiple shoot formation in pepper. The micro shoots could be rooted in MS medium, containing IBA. The success at rooting and planting out stages were 100 and 80 per cent, respectively. Thousands of plants were produced and planted out. Plants were established in farmers' fields in Idukki, Wynad, Thrissur and Kannur districts. The tissue culture plants from different sub-culture cycles were also planted to evaluate their genetic stability. The tissue culture derived plants are performing well even under average management in all the districts. The field establishment varied from 50 to 100 per cent. The established plants revealed normal growth pattern and flowering. More than twenty per cent of the plants started bearing within eighteen months after planting. Abnormalities were not observed for spike and berry characters. No significant variation was observed in the morphological traits, compared to the plants raised through conventional propagules. Tissue culture derived plants produced more laterals, a desirable trait contributing to yield. No distinct variation was observed for the dryage, oil and oleoresin content. (Table1).

Table 1. Varietal performance of tissue culture derived black pepper plants, as compared with plants propagated through cuttings (four years after planting, at the College of Horticulture, Vellanikkara)

Character	Panniyur 1		Panniyur 2		Panniyur 4		Karimunda	
	TC plant	Cutting	TC plant	Cutting	TC plant	Cutting	TC plant	Cutting
Yield (green, kg/plant)	1.5	1.1	0.8	0.8	1.8	1.7	1.8	1.3
Height (m)	2.6	3.2	2.4	3.0	3.0	3.5	2.6	3.7
Internodal length (lateral)	6.0	7.4	5.0	6.0	5.1	6.0	6.6	8.5
No.of laterals	63	38	78	50	93	30	87	30
No. of spikes/ lateral	1.5	1.4	1.8	1.5	2.0	1.5	2.0	1.3
Length of spike (cm)	11.3	11.0	8.3	8.3	7.5	8.2	8.9	7.5
% set	85.7	94.3	83.8	95.7	68.2	88.5	93.2	64.3
Driage (%)	28	32	30	31	32	30	33	33
Oil (%)	1.2	1.1	1.1	1.4	1.5	1.7	1.8	2.0
Oleoresin (%)	11.2	10.6	12.6	13.0	12.5	12.3	12.0	11.5

Cardamom

In vitro techniques for the propagation of cardamom were standardized. The most suitable medium for the establishment of primary shoot buds was MS or SH. For further shoot proliferation half strength MS medium or normal SH medium were suitable. Shoot primordia of 2.5 cm were the best initial explants. For subsequent shoot proliferation, 2.0-3.0 cm long shoots excised from the basal proliferation cultures were used. The plantlets were rooted *in vitro* and successfully established *ex vitro.*

Ginger

Protocol for rapid multiplication of ginger has been standardized. Sprouting buds were identified as the best source of explant for initiating *in vitro* culture of ginger. Of the different basal media tried, MS medium was ideal for ginger. BA 2.5 mg/l was the most effective for getting maximum number of multiple shoots. The yield of rhizomes of tissue culture derived plants was less, compared to the conventional plants raised from rhizomes.

Cashew

Suitable media for raising *in vitro* seedlings, media and growth regulator concentration for induction of establishment, multiplication, elongation and *in vitro* rooting of shoot buds were standardized. The shoot tip, nodal segments and cotyledonary nodes taken from *in vitro* raised seedlings were established after culturing in the dark for seven days on MS medium supplemented with NAA, kinetin, brassinolide and activated charcoal. The explants initiated growth when cultured under light. The cultures were then transferred to MS medium supplemented with 100 ml/l coconut water, brassinolide and activated charcoal for a further period of three weeks. The different varieties showed variation in the number of multiple shoots formed. The addition of brassinolide at low concentration increased the overall vigour of the cultures as well as the number of multiple shoots formed, which ranged from 3.3 for Kanaka to 6.2 for Anakkayam-1. The shoots formed had to be cultured in medium containing lower concentration of plant growth substances for inducing elongation of the shoots. The shoots were successfully rooted in MS medium supplemented with IBA and IAA and by pulse treatment with IBA, followed by transfer to liquid MS medium. Plantlets after hardening have been successfully transferred to the field. Micro-grafting was feasible by adopting side grafting. Such grafts have been successfully hardened and transferred to soil. Explants from adult trees could be effectively surface sterilized by soaking for 60 minutes in Bavistin 0.1 per cent, followed by treatment with mercuric chloride 0.2 per cent for seven minutes to which sodium lauryl sulphate 0.1 per cent had been added. The explants were initially established by culturing on MS medium supplemented with BA 1.0 mg/l, kinetin 0.5 mg/l and activated charcoal 0.5 per cent. Elongation of these shoots was obtained by culturing on MS medium supplemented with kinetin 2.0 mg/l, BA 0.5 mg/l and activated charcoal 0.05 per cent. Further work to induce proliferation of shoots is in progress. Callus could be induced from cotyledonary bits, inter-nodal segments,

leaf bits and nucellus by culturing in media with combinations of auxins and cytokinins. Addition of glutamine and casein hydrolysate increased the percentage of callusing as well as reducing the time needed for callus induction. Profuse root regeneration from the calli was observed when sub-cultured. Shoots with normal morphology could also be induced. Somatic embryoids could be induced in from the nucellus as well as immature cotyledons of cashew cultured on LS, SH, MS and Y3 basal media containing supplements. The induction was favoured by incubating the cultures in darkness initially for two weeks. Shoot and root development was observed from the embryoids on subculture to medium of different composition. The root formation was profuse and the shoots formed were compressed and malformed. Near-normal leaves were produced on subculture. Secondary embryogenesis could also be induced. In another study, germination of cashew somatic embryoids could be induced, in the presence of light, on a combination of basal media with B5 major salts and MS minor salts supplemented with BA 1.0 mgl^{-1}, PVP 10 gl^{-1}, coconut water 200 ml l^{-1}, sodium chloride 0.1 %, cobalt chloride 10 g l^{-1}, sucrose 50g l^{-1} and agar 6.0 gl^{-1}.

Cocoa

Bud culture in cocoa was initiated with the objective of mass clonal propagation of proven genotypes. Nodal segments from field grown trees as well as axenic seedlings were used as explants. Seedling explants showed very good response in bud burst, shoot growth and rooting. These plantlets could be hardened and planted out and field established. They grew, flowered and fruited just like the normal plants. Micro-propagation with explants from field grown trees was difficult. The ideal explant size and sterilization procedure were standardized. Ideal medium was identified for bud burst and shoot growth. Sustained growth of shoots was not satisfactory. Rooting was achieved by pulse treatment with IBA. However, the percentage of rooting was very low. A few plantlets could be successfully hardened and planted out. Micro-grafting was standardized to surmount the rooting problem in cocoa. Somatic embryogenesis was attempted from all tissues but response was obtained only from tender explants. Embryoids were produced directly from cotyledons and their fruit size ranged from 2-5 mm. Larger embryoids germinated to plantlets but their field establishment was not possible. Anther culture was attempted to produce haploids. Callusing could be induced. However, organogenesis could not be induced.

Nutmeg

Nodal segments with a part of the lamina were used as explants. Bud burst from axillary buds was observed two weeks after culture on SH medium supplemented with BA, NAA and activated charcoal. Expansion of the leaf lamina was observed, along with elongation of the shoots up to 2.0 cm. Generally terminal buds responded less than axillary buds. The best and uniform response was observed in explants from grafted plants. Terminal buds responded in WPM medium, supplemented with BA 2.0 mg/l and kinetin 0.5 mg/l. *In vitro* cultures initiated on SH medium supplemented with BA 2.0 mg/l and NAA 1.0 mg/l showed better expansion and retention of leaves

when sub-cultured to liquid SH medium of the same composition, supplemented with activated charcoal 2.0 per cent and sucrose 40.0 g/l. However, in all the cases, growth of the cultures on further sub-culturing was not satisfactory, in spite of the fact that a variety of compounds were tested. Attempts for inducing rooting of the micro-shoots were not successful.

Molecular techniques were tried for sex determination in nutmeg plants. Tender leaves were observed to be the best material for DNA isolation. In the RAPD analysis, eight primers were monomorphic, while three primers yielded polymorphic DNA fragments between the sex. However, the reproducibility of the polymorphism was not satisfactory. Cleaved Amplified Polymorphic Sequence Assay was found to be advantageous for further analysis.

Cinnamon

MS and WPM were used for the culture establishment of nodal segments. WPM showed better response when incorporated with BA 0.5 to 1.0 mg/l or kinetin 0.1 to 1.0 mg/l. Liquid medium, supported with filter paper bridges, favoured better growth of cultures. Multiple shoot formation up to eight per culture was observed in WPM + BA 3.0 mg/l + kinetin 1.0 mg/l. Seedling explants responded better. Seasonal response was observed for leaf expansion and retention. Addition of glutamine 0.5 to 1.0 mg/l favoured leaf expansion and retention. The major problem with the tissue culture of cinnamon is drying of the multiple shoots formed and lack of sustained growth. Rooting could be induced by a quick dip in IBA 3000 mg/l for 15 seconds, followed by transfer to WPM containing 0.5 mg/l each of IBA and IAA. Mortality of plantlets during hardening was high. One hundred and thirty plants from seedling explants could be established *ex vitro*. Efforts for inducing regeneration from the callus were not successful. Conversion of a few somatic embryoids formed was also not successful.

Clove

Systemic fungal infection was the main problem with the culture establishment of clove explants. This could be substantially overcome by pretreatment with different fungicides and by incorporating miconozole in the establishment medium. Shoot multiplication in the range of two to five was observed in the basal media such as MS and WPM when incorporated with cytokinins like BA and kinetin and the auxin IAA at varying concentrations (0.5 to 5.0 mg/l). Multiple shoots could be obtained in WPM supplemented with combinations of different auxins and cytokinins. The ideal combination was identified to be WPM with IAA and BA each at 1.0 mg/l. The proliferation rate was very low in cultures established from mature tree explants. Survival of the multiple shoots in serial sub-culturing was better in liquid shaking cultures. Rooting of the micro shoots could be achieved by a pulse treatment with IBA 5000 mg/l, followed by culture in WPM + IBA 2.0 mg/l + IAA 1.0 mg/l + activated charcoal 0.2 per cent. Rooted shoots could be planted out after six weeks in culture. Most of the plantlets survived the hardening stage and are established well. However,

the slow growth nature of clove was well expressed by the *in vitro* raised plantlets. Post plant out survival was seventy per cent and the plants exhibited normal growth.

Callus could be induced from various explants like inter-nodal segments and shoot tips. MS medium was the best for the induction of callus. The auxins IAA, NAA and 2,4-D favoured callusing. Better callusing was observed when the cultures were sub-cultured to medium containing higher concentrations of auxins. Upon sub-culturing to hormone free basal medium pro-embryogenic masses were observed within one week. Further development could not be achieved. A few somatic embryoids could be induced from leaf callus in half MS medium supplemented with NAA, BA, kinetin, glutamine and casein hydrolysate.

Vanilla

Vanilla planifolia is the natural source of vanillin. There is an increased interest in vanilla cultivation in Kerala in recent years. Vanilla is propagated by stem cuttings of 30-45cm length. There is an acute shortage of quality planting materials. Protocol for the mass *in vitro* clonal propagation of *Vanilla planifolia* was standardized. A multiplication rate of 15 shoots per culture in five weeks could be achieved *via* enhanced release of axillary buds from nodal segments, cultured on MS medium supplemented with BA 1.0 m/l, IAA 1.0 mg/l, sucrose 30 g/l, coconut water 200 ml/l and agar 6.0 g/l. Rooting of shoots occurred in the proliferation medium itself. Large number of plants could be planted out.

Garcinia gummigutta

Multiple shoots could be induced from nodal segments of Malabar tamarind. Rooting of microcuttings was obtained in 60 days of culture. Shoot regeneration could be induced from calli masses derived from 3/4th maturity endosperm. Somatic embryogenesis could be induced from triploid endosperm calli cultures in four months of culture. Methodology could be standardized for isozyme analysis (Esterase) to identify the male and female sex forms at seedling stage. Techniques were also standardized for isolating protoplasts from diploid and haploid tissues.

Capsicum

Protocol for micropropagation has been standardized from hypocotyl segments of *in vitro* seedlings. Hypocotyl segments placed upside down inMS medium with 0.3 mg/l IAA + 5 mg/l BA resulted in production of multiple shoots. Shoot elongation was obtained only for buds that originated from hypocotyls. MS medium with 0.025 per cent activated charcoal was found to be best for shoot elongation of regenerated buds from hypocotyl. Rooting was observed in MS medium with 0.025 per cent activated charcoal.

d. MEDICINAL PLANTS

Kaempferia galanga

Protocol for the rapid multiplication of *Kaempferia galanga* were standardized. Rhizome sprout from mature rhizome was the best explant. Dipping the explants

in 50 per cent ethyl alcohol for two minutes and treating with mercuric chloride 0.1 per cent for seven minutes were effective for surface sterilisation. Murashige and Skoog basal medium at half concentration supplemented with BA 8.0 mg/l was the best for shoot proliferation. Ten to fifteen multiple shoots per culture were produced in a single culture period. The plantlets could be established *ex vitro* in sterile sand contained in poly bags. Field evaluation studies showed that morphological differences existed between tissue cultured plants and conventional plants with respect to leaf orientation, number of tillers, number of roots, root length and number of rhizomes. In tissue culture derived plants, the number of tillers, number of roots, root length, and number of rhizomes and tubers were more than that of the conventional plants. However, the rhizomes were of smaller size. After six months of planting, the girth of rhizomes of tissue culture derived plants was an average of 2.0 cm, whereas that of conventional plants was 8.0 cm. The total yield, dry weight and percentage recovery of oil from the rhizomes were comparable. Axenic cultures were raised from rhizome buds as explant source for callus induction. Pseudostem bit was the best explant for callus induction. Regeneration through organogenesis as well as embryogenesis was obtained from the cultured calli with varying levels of growth regulators incorporated in the culture media. The tissue culture derived plants showed wide variations from the conventionally propagated plants in the field with respect to different morpho-logical characters.

Trichopus zeylanicus

Buds from young purple shoots of mature plants had maximum establishment and growth on SH medium supplemented with BA. Proliferation rate was higher at higher concentration of BA; however, shoot development was better at lower concentration. Addition of adenine sulphate increased the proliferation rate of buds and development of shoots; but supplements like yeast extract and casein hydrolysate were not effective in promoting shoot growth. Tender leaf and petiole explants responded better than mature explants and the percentages of callus initiation and callus index were higher in combination of NAA and 2,4-D with BA. Direct organ ogenesis was observed in half MS supplemented with BA and NAA. Regeneration of healthy and longer shoots was obtained on MS medium supplemented with kinetin. Somatic embryogenesis was observed in media containing BA and BA + coconut water and embryoid germination was obtained on MS medium. Maximum rooting was observed by culturing shoots in media containing brassinolide for one week and thereafter transferring to media containing IBA. Early rooting was obtained in liquid medium. Keeping in half MS with reduced concentration of sucrose and increased light intensity in the culture room for two weeks prior to the transfer to the hardening unit resulted in better survival of plantlets. Plantlets were successfully transferred to soil.

Coscinium fenestratum

Callus could be initiated from leaf segments on half MS medium supplemented with IAA and BA. Callus was also initiated from immature fruits when cultured on

solid half MS medium with phosphate ion reduced to 25 per cent and supplemented with IAA and BA. The shoot tips and nodal segments exhibited recalcitrancy. The cells did not respond to regeneration treatments being neither organogenic nor embryogenic. The wave length of marker berberine was recorded as 228 nm. Berberine was detected in calli produced from leaf explants of different treatments. Age of the callus had a profound influence on berberine production. Incorporation of abscissic acid at 0.25 mg/l sustained the callus development and 0.095 per gram of berberine per gram of callus was recorded. The quantity of berberine detected from field grown plants was 0.013 mg/g leaf and that from the stem was 0.010 mg/g stem. The *in vitro* derived cultures had higher amounts of berberine than the samples from the field grown plants. The highest berberine yield was obtained when phosphorus ion sources were reduced to 25 per cent in half MS liquid medium supplemented with IAA and BA and the recovery was 10.079 mg/g callus.

Gymnema sylvestre

Among the explants, inter node was the most potent in initiating and proliferating calli. Among auxins, maximum callusing in minimum time was achieved by addition of 2,4-D. Combination of 2,4-D with BA showed the highest callus initiation, maximum proliferation and also the fastest growth rate. Production of saponins at two months age of callus was the highest in MS + 2,4-D (2.0 mg/l + BA 1.0 mg/l) yielding 270 mg^{-1} of callus weight. Best medium combination to produce maximum saponins within minimum time was MS + 2,4-D (2.0 mg l^{-1}) + BA(1.0 mg l^{-1}) + malt extract (1.0 per cent) which produced saponins at the rate of 32.75 mg (dry 1^{-1} (tube) $^{-1}$

Holostemma annulare

Rapid multiplication of *Holostemma annulare* could be obtained using nodal explants. Maximum shoot proliferation was observed when the explants were initially cultured on MS medium supplemented with high concentration of BA and later subcultured to the same medium containing lower concentration of BA. Shoots could also be proliferated on MS medium containing extremely low concentrations of TDZ. *In vitro* rooting of shoots could be obtained on MS medium. *Ex vitro* rooting was observed when the shoots were treated with IBA 1000 mg/l (quick dip method) followed by planting in plastic pots filled with sand. Later the plants were transferred to plastic / mud pots containing cocofibe. Embryogenic callus was obtained when the explants were cultured on MS basal medium supplemented with 2,4-D. Leaf segments oriented with the abaxial surface touching the solid medium supplemented with 2,4-D and exposed to light produced embryoids after one or two subcultures on MS medium with lower concentrations of 2,4-D. Somatic embryos were also initiated when the callus was transferred to the liquid medium containing lower concentration of 2,4-D. Elongation of the embryos was observed in solid MS basal medium. Encapsulated beads could be produced by treating the nodal segments with sodium alginate 2.5 per cent and complexing for 30 minutes with calcium chloride 75 mM. The beads could be stored for 15 days at room temperature and up to 40 days at 4°C. The

peroxidase isozyme pattern of the leaves and roots from the *in vitro* and *in vivo* plantlets were similar, suggesting the true to type nature of the plantlets. The study indicates the feasibility of mass multiplication of the crop using *in vitro* techniques.

Tylophora indica

Screening of explants, media and plant growth substances have been carried out for effective shoot bud culture in *Tylophora indica*. Procedures for establishment, proliferation and elongation of shoots have been established. Maximum shoot proliferation was observed when the explants were initially cultured on MS medium supplemented with high concentration of BA and later sub-cultured to the same medium containing lower concentration of BA. *In vitro* rooting of shoots could be obtained on MS medium. *Ex vitro* rooting was observed when the shoots were treated with IBA 1000 mg/l (quick dip method) followed by planting in plastic pots filled with sand. The plants were successfully transferred to soil. Suitable explants, media and growth regulator treatments have been standardized for induction of callus. Direct organogenesis could be induced. Encapsulated beads could be produced by treating the nodal segments with sodium alginate 2.5 per cent and complexing with calcium chloride 75 mM for 30 minutes.

Aristolochia indica

Aristolochia indica is a slender climbing medicinal herb commonly found in the semi-evergreen forests of South India. The active principles of this plant, the aristolochic acids are isolated from roots. The plant is anti-poisonous, anti inflammatory, cardiac and nervine stimulant, carminative, anti-helmintic, blood purifier, expectorant diuretic, diaphoretic and also uterine contractive.

Explants from field grown plants were surface sterilized in 0.1 per cent mercuric chloride for 10 minutes, followed by thorough rinsing with sterile double distilled water. The explants were cultured on half strength Murashigse and Skoog medium without plant growth regulations. After two weeks, live explants free of contamination were sub-cultured into MS media supplemented with BA and IAA at different combinations, *viz*, BA 0.5, 1.0 or 2.0 mg/l and IAA 1.0 or 2.0 mg/l. The buds started emerging and grew out within two to four weeks. The maximum rate of proliferation (53 shoots per culture) was noticed in half strength MS medium containing BA 0.5 mg/l and 1AA 1.0 mg/l at the end of eight weeks. Higher levels of BA in the medium tended to decrease the multiplication rate as well as the maximum shoot length.

Aegle marmelos

Techniques for *in vitro* propagation of *Aegle marmelos* could be standardized with respect to basal media, media components and culture conditions for shoot proliferation *via* enhanced release of axillary buds. Of the two types of explants tried for enhanced release of axillary buds (nodal segments and cotyledons), cotyledons responded better with respect to survival % (100) and shoot proliferation (49 shoots per culture). Nodal segments recorded a maximum of 50% survival and 7.33 shoots

per culture. BA 2.5 $mgl^{-1}$and IAA 1 mgl^{-1} gave maximum multiple shoot proliferation. For enhanced release of axillary buds from cotyledons, BA 0.5 mgl^{-1} registered maximum number of shoots per culture. Hence this was used for standardisation of other media components. However the length of shoots was maximum in the treatments with auxin alone. IAA 2 Full strength MS basal medium mgl^{-1} registered the highest value for the length of the longest shoot (8.25 cm). Full strength MS basal medium was best for shoot proliferation compared to Half strength SH, B5 and WPM. Addition of GA3 3 mgl^{-1} produced lengthy shoots. Supplementation of adenine sulphate at 20 mgl^{-1} improved shoot proliferation. Agar at % mgl^{-1} recorded maximum number of shoots. Among the different plant growth substances tried t initiate direct organogenesis from cotyledon, BA 0.4 mgl^{-1} was ideal for producing maximum number of shoots. Best response with respect to maximum number of shoots was evoked by the combination BA 0.2 mgl^{-1} and IAA 2 mgl^{-1}. Highest callus index was recorded by BA 0.5 mgl^{-1} . Combination of BA 2 mgl^{-1} and IAA 0.5 $mgl^{-1}$was ideal for maximum shoot proliferation from callus. For rooting, IBA was found to be better with respect to early rooting and number of roots. IBA 2.5 mgl^{-1}produced maximum number of shoots (3.33). Among the various basal media tried, earliest root initiation took place in half strength MS and WPM. Maximum number of roots was produced in full strength MS basal medium. Sucrose at 20 gl^{-1} in the rooting medium registered earliest root initiation and maximum length of root while sucrose at 30 gl^{-1} produced maximum number of roots. Among auxins tried for pre treatment of shoots planted out for ex vitro rooting, IBA at 1000 mgl^{-1} for 20 s (quick dip) was best with respect to highest survival rate (75%) after 4 weeks. Sand was ideal potting medium for ex vitro establishment. Embryogenic callus induction in bael was noted in MS basal medium supplemented with, 2,4-D 0.2mg l^{-1}, BA 0.1mg l^{-}, Coconut water 200ml l^{-}, sucrose 40.0g l^{-1} and agar 6.0g l^{-1} . But percentage initiation was low (16.67 per cent). Darkness favoured maximum callus initiation compared to light. The embryogenic calli obtained in the induction medium, when subcultured in half strength MS basal medium supplemented with BA 0.2mg l^{-1}, sucrose 40.0g l^{-1} and agar 6.0g l^{-1} initiated 40 to 50 somatic embryos.

Centella asiatica

Centella asiatica is an important medicinal plant widely distributed in tropical and subtropical regions. It is used in the treatment of various skin diseases, ulceration, chronic rheumatism, leprosy, malaria, epilepsy and enlargement of glands. There is wide variability in the contents of the commercially important chemical compounds of Centella depending on the source / ecotypes of the plants. The glycosides found in Indian plants include brahmoside, brahminoside and minor amounts of asiaticosides which vary in their relative contents depending on the ecotypes. An RAPD analysis of ten accessions, collected from different geographical locations viz., Parumala (Kottayam District), Kottayam, Nagercoil (Tamilnadu), Mananthala (Trivandrum District), Vlathankara (Trivandrum District), Ponmudi (Trivandrum District), Angamaly (Ernakulam District), Punalur (Kollam district), Pattambi (Palakkad district) and

Kozhikode was attempted. Total genomic DNA was isolated from the ten accessions following the protocol of Doyle & Doyle (1990). DNA amplification was studied using forty, decamer primers. PCR amplification involved 43 cycles of the following reaction profile: initial denaturation at 95°C for 3 min, at start, followed by 43 cycles of denaturation at 95°C for 1 min, annealing at 35°C for 1:30 min and extension at 72°C for 2 min. A final extension at 72°C for 5 min was performed after 43 cycles. Four selected primers, which produced reproducible banding patterns, yielded 155 scorable bands with 60 monomorphic bands. An average of six bands were observed. Data analysis was done using NTSYSpc ver.2.02i software. Similarity coefficients were estimated using Nei and Li's coefficient of similarity. The similarity coefficient for the 10 accessions ranged between 0.71 and 0.88. A dendrogram was constructed based on the similarity coefficient matrix. At a similarity coefficient of 0.81, five clusters were formed. Accessions collected from Parumala, Mananthala and Kottayam formed the first cluster. Nagercoil, Ponmudi and Punalur collections formed the second cluster. Vlathankara and Kozhikode collections formed the third cluster. The accessions from Angamaly and Pattambi were grouped under the forth and fifth cluster, respectively.

Smilax zeylanica

Axillary buds from shoot tip and single node explants of *Smilax zeylanica* were induced to proliferate on half strength Murashige and Skoog basal medium supplemented with benzyl adenine (1.0 mgl^{-1}) and indole-3-acetic acid (1.0 mgl^{-1}). Rooting (83.3 per cent) of micro shoots could be induced on the same basal medium supplemented with NAA 0.5 mgl^{-1}. Individual rooted plantlets could be successfully transferred to soil and established in the field.

Plumbago rosea

Plumbago rosea is becoming rare due to the massive collection by medicinal plant traders and also the destruction of their natural habitat. Propagation through seed is unreliable due to poor germination and death of young seedlings under natural conditions. Indirect organogenesis of shoots were obtained from callus derived from leaf and stem explants of *Plumbago rosea* on semisolid half strength Murashige and Skoog's medium. Benzyl adenine (2.0 mg l^{-1}) promoted the proliferation of shoots. The shoots were rooted in MS basal medium supplemented with Indole-3- butyric acid and μ-Naphthalene acetic acid. The rooted plantlets were transferred to pots in a greenhouse and later to the field with ninety per cent survival.

Clitoria ternatea

Clitoria ternatea Linn comes under the family Papilonaceae is popularly grown throughout India. The root is purgative, useful in fever and chronic bronchitis. It is also used as a remedy for hemichrania. The root bark decoction is given as demuleent in the irritation of the bladder and urethra. *In vitro* propagation of *Clitoria ternatea via* somatic organogenesis could be standardized. Callus formation from shoot segments could be induced on Murashige and Skoog medium supplemented with BA and NAA.

Benzyl adenine (1.0 mg l^{-1}) promoted shoot regeneration and proliferation. Napthalene acetie acid (1.0 mg l^{-1}) induced root formation from the microcuttings. The regenerants were transferred to po in a green house and later to the field. 90 percent of the transferred regenerance were survied.

Rubia cordifolia

Bud break and elongation was observed in nodal explants cultured in MS basal medium supplemented with 1 mg l^{-1} BA and 0.2 mg l^{-1} IAA. Rooting of the shoots could be induced in MS basal medium supplemented with IBA 1 mg l^{-1} and plantlets were successfully planted out in 100 days.

Geophila repens

Bud break was obtained in nodal explants cultured in MS medium supplemented with IAA 0.5 mg l^{-1} and BA 1.0 mg l^{-1}. Subculturing shoots from multiplication medium to basal MS medium at half strength, devoid of any supplements resulted in better shoot elongation and leaf development. MS medium at half strength supplemented with IBA 2 mg l^{-1} produced roots in 90 per cent of cultures which could be successfully planted out without any casuality.

Trichosanthes cucumerina

Shoot induction and proliferation of nodal cultures were observed in MS medium supplemented with BA 0.5 mg l^{-1}. Elongated shoots were rooted in half MS following pulse treatment with IBA 1000 mg l^{-1}. Hardening of the rooted cultures in MS medium at half strength supplemented with 2 per cent sucrose for 15 days reduced casuality giving better survival.

Crataeva magna

Bud break and multiple shoot induction was observed in MS medium supplemented with BA 0.5 mg l^{-1} and kinetin 0.5 mg l^{-1}. Rooting could be induced in MS medium supplemented with NAA (0.02 mg l^{-1}). The total duration from culture initiation to plant out was four months.

Stevia rebaudiana

Culture establishment was induced on half strength MS media supplemented with 1.0 mg l^{-1} BA. The rate of multiplication was increased in half strength MS media supplemented with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} kinetin.

Spathyphyllum cannaefolium

Culture initiation was induced on half strength MS basal medium. Further shoot multiplication was induced on half MS supplemented with 1.0 mg l^{-1} BA alone. Both multiplication and shoot elongation was obtained in half strength MS media supplemented with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} kinetin. Rooting was also simultaneously in the same media.

Mycrophyllum aquatium

Culture establishment was achieved on the half strength MS medium supplement4ed with 1.0 mg l^{-1} BA. Shoot multiplication was successful in half strength MS media supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} kinetin.

Aquilaria agallocha

Culture initiation was obtained on half strength MS media supplemented with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} kinetin. Further shoot multiplication was obtained on half strength MS media supplemented with 0.1 mg l^{-1} BA; 0.5 mg l^{-1} NAA and 10 per cent coconut water.

***In vitro* conservation**

In vitro conservation of *Kaempferia rotunda* up to 9 months was possible in MS medium at half strength supplemented with 2.0 per cent sucrose and 4-5 per cent Mannitol. In *Trichosanthes cucumerina* nodal cultures could be conserved *in vitro* for 5 months in MS medium at half strength supplemented with sucrose 2.0 per cent and Mannitol 3.0 per cent.

e. Forest species

Dalbergia latifolia

Axillary bud was identified as the best explant for *in vitro* clonal propagation of Indian rosewood (*Dalbergia latifolia*). The explants could be effectively surface sterilised using mercuric chloride 0.1 per cent for 12 minutes. Both WPM and MS medium are suitable for the establishment of cultures. Phenolic interference could be overcome by series of treatments, including washing in running tap water for one hour, soaking of the explants in ascorbic acid and citric acid (150 mg/l each) for three hours, and incorporation of the same concentration of the antioxidants in the culture medium along with PVP 0.7 per cent. About 25 multiple shoots per culture could be induced on MS medium supplemented with 0.25 mg/l BA along with 1000 mg/l casein hydrolysate. Auxins did not improve the multiplication rate. Sucrose 20 g/l was the ideal source of carbon. Continuous cultures for repeated harvesting of shoots from primary cultures could be developed using MS medium supplemented with kinetin 0.5 mg/l and BA 0.5 mg/l. *In vitro* rooting of the micro shoots could be achieved by pulse treatment with IBA 1000 mg/l and transfer to half WPM with 1.0 per cent activated charcoal. *Ex vitro* rooting was achieved in vermiculite. While 100 per cent rooting could be achieved in vitro, for the *ex vitro* rooting the percentage of success was only 60.0 per cent. Soilrite was identified as the best medium for planting out, supporting about 90 per cent survival of the plantlets. While *in vitro* propagation using explants from young trees could be achieved, micro-propagation of the mature trees (40-50 year old) was difficult. Though, bud break and shoot morphogenesis was observed in MS medium containing BA, kinetin and 2iP (alone or along with IAA or NAA), multiple shoot formation and expansion of leaves could not be induced. L-glutamine was effective in preventing the precocious drop of leaf initials.

Ailanthus triphysa

In vitro propagation of matti (*Ailanthus triphysa*) could be achieved using axillary bud culture. The explants could be surface sterilized by dipping in 0.1 per cent mixture of the fungicides, Bavistin and Indofil for 30 minutes, followed by treatment with mercuric chloride 0.1 per cent for 20 minutes. The phenolics problem could be overcome by including 0.25 per cent activated charcoal in the culture medium. MS medium supplemented with BA 3.0 mg/l and kinetin 1.0 mg/l was the best for supporting multiple shoot formation to the rate of 4.3 shoots per culture. Further elongation of the shoots could be achieved using GA_3 and BA or kinetin. *In vitro* rooting of micro shoots could be achieved in MS medium containing IAA 4.0 mg/l and IBA 0.4 mg/l along with activated charcoal 0.5 per cent. However, the rooting percentage was very low (6.3). There was problem for the successful ex *vitro* establishment of plantlets.

Pterocarpus marsupium

In bijasal (*Pterocarpus marsupium*) micropropagation was attempted using axillary bud explants. Shoot initiation was achieved in hundred per cent cultures in a number of media combinations using MS salts, leaf morphogenesis could not be achieved in most of them. Lack of proper leaf morphogenesis and precautious leaf shedding were the serious problems encountered in bijasal micropropagation. Multiple shoot production with a maximum of up to 5 shoots per culture has been achieved in MS + 3.0 mg l^{-1} BA + 0.5 mg l^{-1} 2ip. These shoots were looking bare either with very small leaves or with no leaf at all in their nodes.

Tectona grandis

Nodal segments collected from mature trees served as the explant for the axillary bud cultures of teak. Among the different media attempted MS media was found to be the most effective with reference to culture response. Shoot proliferation was maximum when the medium was supplemented with kinetin and IAA each at 0.5 mg l^{-1}. Multiple shoot production was achieved when different levels of BA or 2ip was added to the MS medium. Addition of growth supplements like coconut water, activated charcoal, adenine sulphate and casein hydrolysate did not have any favourable effect on growth and establishment of the cultures. *in vitro* rooting of the micro shoots was maximum in ½ MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA + 0.25 per cent activated charcoal along with pulse treatment of the cut ends of the shoots. Further growth of the roots was enhanced when the cultures were transferred to auxin free media after 7 days. Highest rooting percentage and root length was noticed in vermiculite under ex *vitro* conditions. Hardened plants have been planted in the field.

Azadirachta indica

Micropropagation was achieved using axillary bud explants. The most effective media for shoot proliferation was found to be MS supplemented with 1.5 mg

l^{-1} BA + 0.5 mg l^{-1} NAA. Rooting of the micro shoots was obtained under *in vitro* conditions in ½ MS medium supplemented with 1.5 mg l^{-1} IAA. Callus production from internodal segments was achieved in MS medium supplemented with varying levels of auxins.

f. Cost effective methods and devices for plant tissue culture

Attempts were made to develop cost effective methods and devices for home scale adoption of micro-propagation in anthurium. Analytical grade chemicals in the culture medium could not be effectively replaced by laboratory grade chemicals. Confectionary grade sugar cubes could be used to replace extra pure sucrose. However, commercial grade crystal sugar could be used for the purpose. Double glass distilled water could be effectively replaced by rain water, in the medium. Agar could be substituted with playing marbles. Ordinary gold-smith type balance could be used as effectively as the electronic balance. pH indicator paper could be used as effectively as the pH meter. Ordinary bottles and jam jars were found effective in place of expensive borosilicate glassware. Pressure cooker was equally effective as the electric autoclave. Ice box was efficient for storage of culture medium. However, the ice-packing had to be changed every 48 hours to ensure low temperature. Fabricated transfer hood was as good as the laminar air flow chamber in providing contamination free cultures. Natural diffused light was as effective as artificial light in the growth of cultures.

3. *IN VITRO* CROP IMPROVEMENT

Tissue culture techniques have been adopted for the improvement of crops and beneficial strains of microbes. Exploitation of somaclonal variation, *in vitro* mutagenesis, *in vitro* pollination and fertilization, embryo rescue, protoplast culture and somatic hybridization etc. were tried.

a. Somaclonal variation

Black pepper

Attempts were made for inducing somaclonal variation in black pepper and developing screening procedures for *Phytophthora* foot rot resistance, using tissue culture techniques. The toxic metabolites present in culture filtrate of *P. capsici* were non-specific and thermo-stable. Calli were induced on stem and leaf segments of *in vitro* seedlings and basal leaf segments of mature leaves of glasshouse plants of five black pepper cultivars *viz.* Panniyur-1, Karimunda, Kalluvalli, Cheriakanyakadan and Balankotta and *P. colubrinum* in MS medium supplemented with IAA 1.0 mg/l and BA 1.0 mg/l. Based on callus necrosis 7.5 per cent v/v of concentrated culture filtrate (CCF) was fixed as the level that could be withstood by the cultivars. Direct selection of calli did not inhibit the regeneration potential and plantlets could be regenerated from calli screened against *Phytophthora* foot rot in all the cultivars, except Panniyur-1. The regeneration potential of the calli of the different cultivars irradiated with gamma rays was very low. Partial purification of the culture filtrate was attempted. The toxic

fraction present in the culture filtrate could be separated using ion exchanges like Dowex 1 and Dowex 50. None of the regenerated calli clones were found to be completely resistant to *P. capsici* in natural screening. In the artificial inoculation of culture disc of *P. capsici,* highly tolerant plants could be isolated from the unscreened population. Cheriakanyakadan gave more tolerant plants, compared to Kalluvally. Flowering was observed in 32 per cent of the calli clones in the second year of planting.

In vitro screening using the non-specific toxic metabolites of *P. capsici* was not effective in developing disease resistant plants. More number of tolerant plants could be isolated from unscreened population, revealing the possibility of exploiting somaclonal variation as such without *in vitro* stress for *Phytophthora* foot rot resistance screening in black pepper.

Attempt was made to unravel the mechanism behind the foot rot disease tolerance in black pepper by studying the protein pattern and β 1-3 glucanase activity in infected and healthy plants of *Piper nigrum* and *Piper colubrinum*. PAGE and SDS-PAGE analysis of the intercellular protein in infected *Piper nigrum* revealed the induction of additional protein / polypeptide which was absent in non infected / healthy control plants. In *Piper colubrinum*, the species reported to be resistant to the disease, both the healthy control as well as inoculated plants expressed a similar protein of the same molecular weight. This could be a pathogen related protein being expressed during infection in *Piper nigrum* whereas a similar protein is natively present in *Piper colubrinum* which might be giving tolerance / resistance to the species. The β 1-3 glucanase activity in the infected *Piper nigrum* increased by 50 per cent within four days after inoculation with the pathogenic fungi while no significant variation for enzyme activity was observed in *Piper colubrinum* within the same period of time. However, the endogenous enzyme activity measured in *P. colubrinum* was even more than the elevated range in *P. nigrum* indicating its role in the resistance mechanism.

The genetic stability and clonal fidelity of TC black pepper plants was assessed. Sixty decamer primers were screened for amplification of black pepper genomic DNA. Ten primers selected for good amplification were used to screen five varieties of black pepper. Three primers, which showed polymorphism and stability of amplification, were used for analysis of TC plants. Tissue culture regenerants derived by bud culture were subjected to RAPD analysis using three primers (OPP-1, OPP-8, OPP-14). All the regenerants studied gave a uniform RAPD profile except in two regenerants where there was difference in expression of two non-distinct bands.

Ginger

Protocols for *in vitro culture,* callus induction and indirect organogenesis were perfected for ginger. Pseudostem base and sheathy leaf base were the best explants for induction of calli. Higher levels of 2,4 D favoured callusing. Regeneration was observed in presence of higher levels of BAP. Silver nitrate favoured indirect organogenesis in ginger. The regenerants were normal and healthy. Good field

establishment was observed for the regenerants. Yield during the first year was comparatively low. Wider variation was observed for yield and disease infection and five variants identified were put to further field trial.

Three liquid media were identified for the *in vitro* production of toxic metabolites by *Pythium aphanidermatum*. Standardisation of the incubation period and culture conditions for the maximum accumulation of toxic metabolites using the three liquid media is in progress. Standardisation of the *in vitro* screening techniques using toxic metabolites of *Pythium aphanidermatum* is also in progress.

The procedure for *in vitro* irradiation in ginger was standardised. *In vitro* rhizome sprouts were identified as the best explant for irradiation. The highest dose was identified as 2.0 kr. The regenerants from irradiated cultures varied for vigour and tiller number. The media suitable for sustained growth in serial sub-culturing was identified. The irradiated plantlets were successfully hardened and planted out. The field performance varied greatly among the plants within the treatment and between treatments. The few regenerants identified as superior were further put for field evaluation.

Rice

Rice varieties were screened for their efficiency for callus production. *In vitro* regeneration from rice calli was standardized. Blast and sheath blight fungal cultures were prepared and tested for their pathogenicity and the work is in progress to initiate disease resistant calli cultures.

Tomato

Protocol was standardized for callus induction, and organogenesis in tomato, cultivar Sakthi and in *L. pimpinellifolium*. The leaf segments exhibited high callus index in the medium supplemented with NAA (2 mg l^{-1}) and BA (1-3 mg l^{-1}) for Sakthi and *L. pimpinellifolium*. The per cent morphogenic capacity was highest in internodal calli followed by leaf segment calli in Sakthi when basal medium supplemented with NAA (2 mg l^{-1}) and BA 3 mg l^{-1}) was used. Screening of somaclones of Sakthi and *L. pimpinellifolium* for resistance to TLCV disease, transmitted by white fly *Bemisia tabaci* has revealed that two somaclones of Sakthi were free from the disease and five plants exhibited mild symptoms. Two somaclones yielded higher than control, another two plants were free from fruit cracking. The somaclones of *L. pimpinellifolium* were free from TLCV disease.

***b. In vitro* pollination and embryo rescue**

Ginger

In ginger, *in vitro* placental pollination with pollen grains suspended in ME3 medium was best with maximum ovule swelling and maximum percentage of cultures with developed ovules (90%). Controlled selfing and crossing among autotetraploids and diploid cultivars using *in vitro* placental pollination were successful. However, the seeds failed to germinate. Somatic embyoids could be induced from

seeds cultured in media combinations of half MS + 2,4-D 0.5mgl^{-1}, BAP 6.0mgl^{-1} and GA3 5mgl^{-1} along with 3% sucrose and also in half MS + 2,4-D 0.1mgl^{-1}, BAP 8.0mgl^{-1} along with 3% sucrose in crosses between diploids and tetraploids.

Pollen fertility and viability in ginger were influenced by season and genotype. However, the position of flowers in the inflorescence was not important. Pollen viability was high in the inflorescence produced during early and mid period of the flowering season. The cultivars SG-66 and Rio-de-Janeiro exhibited more pollen fertility and viability. Chances of seed set would be more in crosses involving these cultivars as male parents. *In vitro* fertilized ovules developed into mature seeds in half strength MS medium supplemented with different combinations of auxins and cytokinins. One seed germinated and developed into plantlet. Primary treatments like water soaking and incubating on moist filter paper, moist sand or basal medium (both liquid and solid) did not favour germination of ginger seeds. Incubating the seeds in medium containing different combinations of auxins and cytokinins also did not favour germination. Seed treatments like chemical and mechanical scarification, stratification, washing the seeds in running water and subjecting to stress condition by dehydrating hydrated seeds for 12 h or soaking the seeds in 12 per cent each of mannitol and PEG - 4000 solution did not influence germination. Embryo rescue studies at 20, 40, 60 and 80 DAP did not produce germination.

Okra

Okra is highly susceptible to yellow vein mosaic. As resistance is not available in okra, inter-specific crosses were made between *Abelmoschus esculentus* and *A. moschatus* (resistant to YVM) to develop yellow vein mosaic resistant cultivars of okra. Post-zygotic incompatibility was found to operate between the species. Embryo culture was attempted to obtain plants from the inter-specific crosses. Twelve to fifteen days old embryos responded *in vitro*. Transplantable seedlings were obtained in MS medium supplemented with BA 0.5 mg/l and coconut water 150 ml/l. Preliminary graft transmission studies indicated that the inter-specific hybrid is resistant to YVM virus.

c. Exploitation of induced variability for crop improvement

Ginger

Two autotetraploids developed through colchicines treatment, Z-0-78 from the cultivar Himachal Pradesh and Z-0-86 from the cultivar Rio-de-Janeiro formed the base material for the study. Evaluation of the autotetraploids along with diploids indicated that autotetraploids are ideal pollen parents with high pollen fertility, viability, pollen size, and pollen tube length. Though *in vivo* pollinations failed, controlled selfing and crossing among autotetraploids and diploids cultivars using *in vitro* placental pollination with pollen grains suspended in ME_3 medium were successful and influenced ovule development. Culture establishment and ovule development was maximum in MS medium at half strength supplemented with NAA

(0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) along with CH (200 mg l^{-1}) or CW (25%) v/v. Semi solid medium was advantageous in obtaining maximum seed set and size at maturity. Mid season of flowering favoured maximum culture establishment and ovule development compared to early and late season in both selfing and crossing. Crossing enabled production of maximum number of developed ovules compared to selfing. The embryos were viable as indicated by the tetrazolium staining. Seed germination studies showed that seeds from crosses between diploid x autotetraploid, autotetraploid x diploid and diploid x diploid germinated showing radicle emergence. Development of somatic embryoids were observed from seeds cultured in media combinations of half MS + 2,4-D 0.5 mg l^{-1}, BAP 6 mg l^{-1} and GA_3 5.0 mg l^{-1} and also in half MS + 2,4-D (0.1 mg l^{-1}) and BAP 8.0 mg l^{-1}.

Turmeric

In vitro pollination in turmeric was done by pollen grains suspended in modified ME3 medium. Ovule/seed development was observed in intra ovarian, placental and modified placental pollination techniques. Half strength MS medium containing 3% sucrose, 1mgl^{-1}BAP, 1mgl^{-1}Kinetin and 0.5mgl^{-1}NAA was the best medium for increasing the size of the ovules. Two hybrids from the *in vivo* crosses (VK70 x VK76) germinated under *in vitro* on moist filter paper in test tubes. They were multiplied under *in vitro* and six plantlets were successfully planted out in the field. Pollen viability was low during early flowering season (18.88%), compared to mid (25.05%) and late (25.84%). Natural fruit set and seed set were observed among short duration cultivars but not among medium duration cultivars.

Anthurium

Six commercially important varieties of *A. andreanum* viz., Nitta, Candy queen, Lima, Red Dragon, Eureka Red, Agnihothri and three species of Anthurium viz., *A. crystallinum, A. ornatum* and *A. amnicola* were selected for the genetic improvement studies by *in vitro* and *in vivo* methods. Out of the 42 combinations of hybridization tried 17 were found compatible. All the self crosses and interspecific crosses were found incompatible. Among all the combinations, Lima produced the largest number of compatible crosses as well as high seed set and germination percentage. The varieties 'Candy Queen', 'Red Dragon' and 'Eureka Red' also performed well as good female parents. Out of the 17 successful combinations, the highly compatible crosses were Candy queen x Lima, Lima x Red Dragon, Lima x Eureka Red, Eureka Red x Red Dragon.

The protocol for immature hybrid seed culture in anthurium was developed. Seeds, 40-45 days before field maturity could be used for *in vitro* culture, thus reducing the time lag for the production of hybrid seedlings. Germination and further development were good in ½ MS + 1 mg l^{-1} BA. For callus initiation, ½ MS with BA 6 mg l^{-1}, NAA 3 mg l^{-1} was effective for rooting and growth enhancement ½ MS with BA 0.5 mg l^{-1} and IAA 1 mg l^{-1} proved good. Irradiation of seeds reduced germination

percentage and further growth. Callus irradiation at 0.5 to 10 Gy was not effective. Lower dosages induced pale green leaves and clustered appearance while higher doses induced browning of cultures. When immature seeds were cultured *in vitro* plantlets could be transferred to the field by 6-7 months.

Tuberose

Investigations on *in vitro* propagation and genetic improvement of tuberose were carried out to develop techniques for *in vitro* regeneration of commercial varieties of tuberose viz., Single, Double, Shringar and Suvasini from different explant sources and attempt to create variability through mutation breeding for commercial exploitation. The scale stem sections from bulbs were found to be the ideal explant for the enhanced release of axillary buds. Inflorescence segments containing immature flower buds were ideal for somatic organogenesis, whether direct or callus mediated. Early release of buds and further growth of buds were better in MS medium supplemented with BAP 6 mg l^{-1} + kin 4 mg l^{-1} in all the varieties. Elongation of multiple axillary buds was obtained in half strength MS medium devoid of growth regulators. The elongated shoots produced maximum number of roots in MS medium supplemented with IBA 4 mg l^{-1} + 0.2% activated charcoal. Field performance of plants derived from tissue culture was comparable with the plants produced by conventional methods. Direct organogenesis could be obtained from immature inflorescence segments in MS medium supplemented with NAA 0.2 + BAP 2.0 + kin 1-3 mg l^{-1}. Inflorescence segments with immature flower buds were the most ideal for callus initiation growth and differentiation.

***d. In vitro* mutagenesis**

Tuberose

For *in vitro* mutagenesis, the safest dose of irradiation at culture establishment stage, shoot proliferation stage and callusing stage were 15 Gy, 15 Gy and 10 Gy respectively. EMS at 1.0 and 2.0 per cent and gamma rays at 15 Gy and 20 Gy were most effective in creating variability. Morphological variants like chlorophyll mutants/ branched flower stalk mutants, compact inflorescence mutants and non flowering mutants were observed at different levels of mutagens. Based on growth parameters and floral characters, nine mutants were isolated viz., dwarf mutants, high tiller mutants, non-tillering mutants, compact inflorescence mutants, tall mutants, long leaf mutants, broad leaf mutants, large flower mutants and large inflorescence mutants. High estimates of heritability coupled with high genetic gain were noticed for number of flowers/spike, spike length, flower diameter, leaf length and leaf width which indicate that the observed variability is heritable and that there is considerable scope for genetic improvement with respect to these traits.

Orchid

In vitro mutagenesis in the orchid Dendrobium variety sonia 17 was attempted. Meristem cultures were established on Vacin and Went medium supplemented with

BA 1.0 mg l^{-1} and NAA 1.0 mg l^{-1}. Protocorm like bodies (PLBs) were established on half strength MS medium supplemented with BA 1.0 mg l^{-1} and coconut water 7.5%. The PLBs were subjected to gamma irradiation. The optimum range was identified to be 2.0 to 3.0 kR. After irradiation the PLBs were proliferated on half strength MS medium supplemented with BA 1.0 mg l^{-1} and coconut water 7.5%. Elongation of the shoots was induced on half strength MS medium supplemented with BA 0.2 mg l^{-1}, NAA 1.0 mg l^{-1} and coconut water 15%. Rooting of shoots was achieved on half strength MS medium supplemented with NAA 1.0 mg l^{-1} and coconut water 15%. Plantlets were established outside. Further observations are in progress.

e. Protoplast culture

Piper spp

Protocols for the isolation of protoplasts from *Piper nigrum* and *P. colubrinum* were standardized. 1.4 per cent cellulase and 0.34 per cent pectinase for *Piper nigrum* and 1.0 per cent cellulase and 0.217 per cent pectinase for *P. colubrinum* were the optimum level of cell wall digesting enzymes when treated for 21 hours in presence of 0.6 M osmoticum. Filtration centrifugation technique was found to be superior in purifying the protoplasts, compared to sucrose floatation method.

Mango

Effort was made for standardizing techniques for protoplast culture in mango. Callus induced from nucellus of immature fruits (30-45 days) was the best plant material. Onozuka R-10 and Macerozyme R-10 were the best enzyme combination with an optimum concentration of 1% and 0.5%, respectively. The highest yield (101 protoplasts per field) was recorded after four hours of incubation. Pre plasmolysis of the plant was not beneficial. Mannitol 9.0 per cent was the best for osmotic regulant. Temperature of 28°C was the best for the highest yield of protoplasts. MS medium with half strength major salts supplemented with BAP 3.0 mgl^{-1}, and NAA 1.0 mgl^{-1} was the best for culturing the protoplasts. Medium containing glucose 90 gl^{-1} as osmoticum supported the formation of cell wall on the 4th day, cell clusters on 15th day and microcalli after four weeks. The best result for cell division and microcalli formation was observed for the treatment involving sucrose 70 gl^{-1}, mannitol 10 gl^{-1}, and inositol 10 gl^{-1}.

Centella asiatica

Successful methods could be standardized for protoplast culture in centella asiatica. Microcallus production could be obtained.

***Fusarium* spp**

Techniques could be standardized for protoplast culture in *Fusarium* spp useful for biological control of water hyacinth. Attempt for protoplast fusion for strain improvement is in progress.

4. GENETIC TRANSFORMATION

Black pepper

Genetic transformation in black pepper variety Panniyur I using *Agrobacterium tumefacins* strain AGL 1.1303 harbouring two antibiotic resistant genes NPT II and HPT IV and GUS and GPF reporter genes were carried out. Callus induction on cotyledonary leaf explants and callus growth were found to be maximum in ½ MS with 1 mg l^{-1} of IAA and BA. Embryogenic calli were not generated. Callus induction on cultures was completely inhibited at 50 mg l^{-1} of kanamycin and 100 mg l^{-1} of hygromycin. Cefotaxime 500 mg l^{-1} effectively killed the bacteria. Leaf transformation was carried out by varying different factors affecting transformation. But ineffective elimination of *Agrobacterium* was obtained. Transformation experiments are also being undertaken with different cultivars of black pepper viz., P_1, P_4 and P_6 and also with different *Agrobacterium* strains.

Ginger

Agrobacterium tumefaciens strain EHA 105 with gus construct was used for standardizing the optimum conditions for effecting transformation in ginger. Co-cultivation period, preculture of explants, bacterial dilution, infection time etc. were standardized. The experiment indicated that a suitable transformation protocol would include 3 days of preculture of explant, a bacterial dilution of 1:20 (v/v), infection time 5 minutes, co-cultivation of 48 hrs and post cultivation on callus induction medium with 100 mg l^{-1} kanamycin + 300 mg l^{-1} cefotaxime in darkness for 4 weeks, followed by 16/8 h photoperiod. This was based on the transient expression as number of GUS expressing units per explant. An efficient protocol for induction of embryogenic callus and plant regeneration was standardized using young bud explants of ginger as a prerequisite for transformation. MS medium supplemented with 2,4-D 1mg l^{-1} and BA 0.5 mg l^{-1} was ideal for embryogenesis in ginger. Shoot and root differentiation were obtained on MS medium with BA 2 mg l^{-1}.

Holostemma

Hairy roots were induced by infection of *Holostemma* explants with a gram negative soil bracterium, *Agrobacterium rhizogenes*. Among the explants tried for hairy root induction seedling hypocotyls showed highest potential for hairy root induction followed by shoot buds. Different strains of *Agrobacterium rhizogenes* viz., P_cA_4, 15834, A_4, 8196 and 2659 were evaluated for their ability to induce hairy roots in Holostemma explants. The strain P_cA_4 showed the highest potential for hairy root induction, followed by strains 15834 and A_4. Direct inoculation of bacteria on wounds induced hairy roots on seedling hypocotyls only whereas co-culture of wounded explants with bacteria induced hairy roots on seedling hypocotyls and shoot buds. Factors like nature of bacterial inoculum, intensity of bacterial inoculum, co-culture time shaker speed and photo period influenced the transformation. Low intensity of bacterial inoculum, long co-culture period, shaker speed of 100 rpm, and a photo-period of 16 h light was found to be the best for hairy root induction in Holostemma.

Hairy roots were induced in a period of one to four weeks. Full strength MS medium favoured hairy root induction while ½ strength MS medium did not favour hairy root induction. The induced hairy roots showed altered phenotypes. The hairy roots obtained directly from explants without NAA treatment were whitish, hairy and showed negative geotropism. The hairy roots obtained after NAA treatment were brownish yellow and were induced from calli formed on wounds after infection. Hairy roots obtained on infection with strains P_cA_4, 15834 and A_4 showed the presence of agropine confirming their transformed nature.

Capsicum

Agrobacterium mediated genetic transformation was attempted with leaf discs and hypocotyl of bell pepper. The time of infection of leaf discs was standardized with gus construct. Three infection times *viz.* 5, 10 and 15 minutes were tried and 10 minutes was found optimum. Two dilutions of bacterial suspension (EHA 105 with gus construct) were tried. Leaf discs of *in vitro* raised capsicum seedlings were precultured for 3 days before infection with *Agrobacterium* strain EHA 105. Cocultivation of the infected leaf discs done for two days. Cultures were transferred to pre selection medium after washing with antibiotics containing liquid MS medium. After one week these were transferred to selection media containing kanamycin (100 mg l^{-1}). Transformation was confirmed by formation of blue spots on staining with X-gluc.

Orchids

The requirements for successful genetic transformation in *Dendrobium* were optimized to evolve a protocol. Protocorms and protocorm like bodies (PLBs) were used as explants. The sensitivity of these explants to various antibiotics *viz.*, a mpicillin, rifampicin, cefotaxime, carbenicillin, kanamycin and hygromycin was evaluated. The explants were sensitive to kanamycin 100mgl^{-1} and hygromycin 50mgl^{-1}. The transformation efficiency (2.0) was high when the infection was made with bacterial pellets, obtained by centrifugation of the overnight grown bacterial suspension. Transformants (1.0 per cent) were obtained with infection for 15 minutes, using bacterial pellets. Transformants (2.0 per cent) were obtained only with the wounded PLBs of 0.2 cm size, co-cultivated in dark for two and three days. The transformation efficiency was increased to 3.0 per cent when acetosyringone 100ìM was added. Maximum transformants were recovered with the strain EHA 105. Among the antibiotic resistant PLBs, 69.23 per cent were positive for GUS assay and the expression was expressed as blue spots and blue patches on the surface of PLBs. Further, PCR confirmation of transformation was also done.

5. MOLECULAR CHARACTERISATION

a. RAPD / AFLP analysis

Black pepper

Genomic DNA was isolated from 49 varieties of *Piper nigrum*. Twenty selected decamer primers were used to amplify the DNA for RAPD analysis. The 44

varieties of *Piper nigrum* were grouped into basically 11 groups with NE collection forming a group totally separate from all others. P1 and P3 which are hybrids of the same parents Uthiramkota x Cheriyakaniyakadan are seen grouped togrther. Similarly, Sreekara and Subhakara are clonal selections from Karimunda in the dendrogram. The other varieties are grouped in to several clusters. P2 (OP progeny of Balankotta) and P4 (clonal selection from Kuthiravally) are grouped together, as also Ayimpiriyan and P5 (OP progeny of Perumkodi).

Genomic DNA of 49 black pepper varieties were isolated and purified for AFLP. However the results showed slight concurrence with those obtained for RAPD markers. The NE collection 3089 is found to be very distinct in both sets of analysis. Varieties Sreekara and Subhakara revealed close relationship in RAPD and AFLP analysis. The variety Uthiramkotta was found to cluster with sager local in both the analysis. Nine *Piper* species were subjected to RAPD analysis with 20 selected primers and AFLP analysis with 12 primer pairs. A total of 148 markers were detected from the RAPD profile and 1005 markers were observed with primer pairs studied in AFLP assay for the 9 species studied. The dendrograms generated for RAPD and AFLP analysis showed a clear cluster formation excluding the species *P. arborium* and *P. colubrinum*, which showed high degree of dissimilarity among themselves as well as within the species studied. They are known to be two exotic species, morphologically very distinct from the other *Piper* species. The genetic diversity observed in the present study between these two species agrees with their existing morphological classification.

Characterization of piper species was done through RAPD and AFLP assay. Nine piper species were subjected to RAPD analysis with 20 selected primers. They were subjected to AFLP analysis with 12 primer pairs. A total of 148 markers were detected from the RAPD profile and 1005 markers were observed with primers pairs studied in AFLP assay for the 9 species studied.

Cashew

Study was taken up with the main objective to fingerprint cashew genotypes based on genetic analysis carried out and the genetic relationship deduced between the morphological, biochemical and molecular parameters studied and also to identify genetically diverse genotypes among those selected for the study to be used in breeding programmes. Molecular studies involved RAPD analysis using four primers which gave 44 amplification products out of which 30 (68.19 per cent) were found to be polymorphic. Two primers OPP-5 and OPP-10 could distinguish varieties Mdk-2 and Mdk-1 with amplicons 22 and 25 respectively. Dendrogram constructed based on the study grouped together Kanaka and Dharasree; Mdk-1 and Mdk-2 and H-1600 and P-302 with the latter two being the closest of all.

Curcuma aromatica

Eleven accessions of Curcuma aromatica, two accessions of turmeric and two accessions of zedoary were subjected to RAPD analysis for comparative study. Forty

primers (OPA and OPB series) were screened. Three primers, found promising were used for the analysis.

Banana

Fifty five distinct cultivars / clones identified based on morphological characterization in the different genomic groups AA, AB, AAA, AAB and ABB representing the genetic diversity of banana in Kerala are being subjected to RAPD assay using 10 decamer primers. The RAPD profile obtained is being utilized to develop markers and to classify varieties.

Twenty eight clones / inctraclones of banana belonging to six genomic groups were collected from different parts of Kerala and Tamil Nadu and subjected to field evaluation and RAPD analysis. Sixty primers were screened and the most promising five primers were used for the analysis.

Eleven ecotypes of Nendran banana collected from different parts of Kerala were subjected to field evaluation and RAPD analysis. Forty primers were screened and the most promising five primers were used for the analysis.

Mango

Two hundred trees of traditional mango varieties of southern Kerala are being evaluated based on morphological and RAPD profiles. Sixty primers have been screened and the most promising ones are being used for the study.

Cucumber

Forty landraces of *Cucumis melo* L. were characterised using morphological, biochemical and molecular markers. Morphological studies showed significant differences among the genotypes for all growth and yield components. Molecular characterization gave a perfect differentiation of dessert melon from culinary melon which is in agreement with morphological characterization.

Ashgourd

Twenty five landraces were characterised and catalogued. The study revealed that morphologically distinct and superior lines are genetically differentiable. RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group.

Ivy gourd

Twenty five landraces were characterised using morphological and molecular markers (RAPD). Molecular characterization gave more precision than the morphological characterization. A superior landrace collected from Kazhakootam, Thiruvananthapuram produced distinct bands in RAPD analysis.

Moringa

Twenty eight accessions of drumstick were collected from different regions of Kerala. Morphological characterization was done on the basis of vegetative, flower-

ing, fruiting and quality components. Molecular characterization was done using RAPD markers and were grouped into five clusters. A superior accession MO 28 produced distinct bands in this analysis.

***Pandnus* spp**

Studies on morpho molecular characterization and evaluation of Pandanus spp have been carried out. Wide variability was observed among the 30 genotypes of Pandanus for morphological characters *viz.*, stem, leaf and spine growth characters and anatomical characters such as leaf thickness and number of stomata. Using Random amplified polymorphic DNA primers, the thirty genotypes were distinctly differentiated by four primers viz., OPB-11, OPB 12, OPB-18 and OPB-20. Jaccards similarity coefficient value ranged from 0.333 to 1.000. UPGMA based dendrogram constructed using similarity matrix data, grouped the 30 genotypes into eight clusters.

Teak

Genetic diversity in natural teak populations and teak provenances of the Western Ghats region were estimated through AFLP technique. DNA was extracted from leaf samples collected from two natural populations of teak, Thamaravellachal located in Peechi-Vazhani wildlife sanctuary and Parambikulam wildlife sanctuary. The samples were collected from 20 randomly selected trees. AFLP analysis for samples from Thamaravellachal was completed. A total of 467 markers were observed with 10 primer pair combinations among 20 plants of the Thamaravellachal population. The dendrogram generated from AFLP banding profiles revealed that the genotypes were grouped into eight clusters. The intra population variability varied from 20 to 40 per cent.

Ralstonia solanacearum

Ralstonia solanacearum strains infecting ginger collected from ginger growing traits of Palakkad, Ernakulam and Wynad were characterized at cultural, biochemical and molecular levels. The isolates were grouped into races based on hypersensitive reaction on capsicum and all the isolates were found to belong to race 3, which was confirmed by DNA amplification using OPF 8 primer. The plasmid profiles of all isolates showed two distinct bands of approximately 21 kb size indicating the possibility of presence of two plasmids. The plasmids, when transformed to *E. coli* DH 5x competent cells imparted resistance to both ampicillin and rifampicin, the antibiotics to which native *R. solanacearum* isolates were resistant. This indicated the possibility of location of antibiotics resistant given on plasmids. Genomic DNA extracted from all isolates were of good quality and quantity. The quantity ranged from 2 to 7.5 μg/ml. RAPD analysis using 16 primers revealed polymorphism among the isolates. Primers Opu 13, Opu 17 and Opx 9 yielded 100 per cent polymorphism. Dendrogram constructed using the data showed two clusters, one major and one sub cluster. Palakkad isolates formed a single cluster indicating similarity among themselves.

***Ralstonia solanacearum* causing bacterial wilt in solanaceous vegetables**

Nine isolates of *R. solanacearum* collected from 3 different locations from brinjal, chilli and tomato were used in the study. The isolates were characterized by cultural, morphological and biochemical tests. Plasmid DNA profile of the isolates were studied and no difference was found in the plasmid DNA profile. Polymorphism among the isolates was studied using RAPD with ten decamer primers RAPD profiles exhibited great diversity among biovars 111 and 111A as well as among race 1 isolates. Race 3 isolates were less polymorphic with certain primers tested. OPF 8 yielded a unique band specific to race 3 isolates. Dendrogram obtained from the pooled data of RAPD profiles also showed high genetic similarity between race 3 isolates.

Fusarium pallidoroseum

The black pea aphid *Aphis craccivora* Koch. is a serious pest of cowpea and several other pulse crops. The nymphs and adults of this pest suck sap from tender shoots, flowers and pods leading to stunting of plants and malformation of pods, causing substantial yield reduction. This also acts as a vector of viral diseases like rosette, mottle, stunt and stripe of pulse crops. Hareendranath *et al.* (1987) reported *F. pallidoroseum* as a fungal pathogen of cowpea aphid *A. craccivora*. Mathai *et al.* (1999) found it an effective biocontrol agent for the management of cowpea aphid. This has been observed as a non-plant pathogenic fungus. Characterisation of the fungal strain seemed important in order to ensure its non-plant pathogenic nature. DNA isolated from the fungus was subjected to RAPD analysis. Twenty primers (10 primers each from Operon A and B series) were used for RAPD amplification. Amplification was observed for five primers. The number of bands per primer ranged from two (OPB 17) to six (OPA 3). OPB 1, OPB 7 and OPB 15 recorded three, five and four bands, respectively. The average number of bands per primer recorded was four.

b. Karyomorphology and isozyme variations

***Piper* spp**

Eleven species of *Piper* were studied for three enzyme systems *viz.* esterase, peroxidase and GOT. Based on the isoenzyme variation nine species could be grouped into three groups. *P. nigrum, P. pseudonigrum, P. barberi* and *P. galeatum* formed the first group with similarity index ranging from 0.38 to 0.78. *P. argyrophyllum* and *P. attenuatum* formed the second group with similarity index reaching up to 0.72. The third group includes *P. chaba, P. hapnium* and *P. colubrinum* with similarity index 0.30 to 0.51. *P. longum* and *P. betle* stood individually showing their distinctness from rest of the species. The least similarity was observed between *P. colubrinum* on one side and *P. pseudonigrum* and *P. barberi* on the other (S.I 0.05).

***Acacia* spp**

Among the four species of acacia studied, two species namely, *A. mangium*

and *A. auriculiformis* possessed 2n-26 chromosomes, while *A. nilotica* is a tetraploid carrying 2n=52 chromosomes. *A. ferruginea*, the species for which the chromosome studies were conducted for the first time, also was found to have 2n=26 chromosomes. Isozyme patterns of esterase and glutamate oxaloacetate transminase (GOT) were analysed in these species. Chromosome studies conducted in these species along with the isozyme patterns obtained suggested that *A. auriculiformis* and *A. mangium* are closely genetically related whereas a. *ferruginea* and *A. nilotica* are distinctly different. Based on the available evidences it is also postulated that *A. mangium* is evolved from *A. auriculiformis* relatively recently.

Areca catechu and allied species

Twenty four arecanut accessions, comprising of four species, five released varieties, 12 traditional cultivars and three exotic cultivars collected from various states were included in the investigation. The isozymes studied were peroxidase, esterase and polyphenol oxidase. The species viz., *A. catechu, A. triandra, A. lutescens* and *A. normadiyii* were displayed variations with respect to the isozyme banding pattern while no variation was observed among the five released varieties. The protein content of the accessions ranged from 3.4 to 8.8 mg/ml extract. The protein content was very high in the YLD tolerant accessions when compared to the susceptible genotype. High protein content along with low enzyme activity levels recorded invariably by all the tolerant accessions points towards a possible influence of the biochemical parameters on YLD tolerant reaction.

Curcuma spp

Isoenzyme banding pattern of 39 genotypes of *C. longa* was studied for esterase, peroxidase and GOT. The similarity index among the genotypes ranged from 0.39 to 1.0. The genotypes were classified into 21 groups with similarity index 1.0 among the members. Morpho-types M-1, M-2 and M-3 formed one group with similarity index 1.0. M-7 and M-8 were also grouped into one. Released varieties under study showed more similarity with first group of morphotypes. On grouping the 18 species of *Curcuma*, based on isoenzyme similarity, *C. zedoaria, C. aeruginosa, C. malabariin, C. comosa* and *C. caesia* formed one group. *C. amada, C. sylvatica, C. aromatica* and *C. harita*, the second group and *C. soloensis* and *C. montana* the third group. Cultivated type *C. longa* and other sessile tuberising species *C. latifolia* and *C. raktakanta* showed variable similarity with other species and stood independently. Stoloniferous type *C. vamana* showed more similarity with sessile tuberising species than with non-sessile tuberising species. Non-sessile tuberising species *C. aurantiaca* and *C. pseudomontana* stood individually, showing their distinctness from the rest of the species.

6. ISOLATION AND CHARACTERIZATION OF PR PROTEINS

Efforts were concentrated for isolation and characterization of PR proteins associated with *Phytophthora* foot rot tolerance in black pepper. Susceptible variety

Panniyur-I, relatively tolerant variety, Kalluvally and the resistant species *Piper colubrinum* were included in the study. Two distinct PR proteins of cationic nature (8 kD and 16.5 kD) were detected in black pepper to be associated with phytophthora foot rot tolerance. ß-1,3-glucanase was identified as one of the PR enzyme induced/ over expressed in black pepper during phytophthora infection. The activity of this enzyme in the tolerant variety Kalluvally and resistant species *Piper colubrinum* was found to be too high compared to susceptible varieties. The expression was confirmed through western blot analysis.

7. CLONING AND CHARACTERIZATION OF GENES ENCODING DEFENSE PROTEINS

DNA sequences encoding three defense proteins viz. ß-1,3-glucanase, hydroxyl methyl glutaryl CoA reductase (hmgr) and chitinase have been cloned from different crop species as well as wild plants. For this, degenerate primers were designed based on conserved boxes in several plant species, already available in databank. These primers were used for synthesizing the gene through reverse transcription, from total RNA. These cDNAs were cloned in plasmid vector and sequenced. Sequence analysis was carried out through multiple sequence alignment (Clustal W 1.8). Details of the genes cloned are given in Table 2.

Table 2. Details of the genes cloned

Sl.No.	Name of plant species	Gene	Size of cDNA cloned
1	*Piper nigrum*	ß-1,3-glucanase	680 bp
2	,,	hmgr	660 bp
3	,,	chitinase	800 bp
4	*Piper colubrinum*	ß-1,3-glucanase	680
5	,,	hmgr	660
6	,,	chitinase	800
7	*Cocos nucifera*	ß-1,3-Glucanase	700
8	*Solanum xanthocarpum*	hmgr	800

Three sequences have been deposited in databank and others are under processing. Full length genes could be isolated and used for imparting biotic stress tolerance to crop species.

8. *IN VITRO* PRODUCTION OF SECONDARY METABOLITES

Sida spp.

Leaf stem and root callus cultures of four species were established *in vitro*. Half strength MS medium supplemented with 2,4-D 1 mg l^{-1} was ideal for initiation and proliferation of calli. Among the species tested, *Sida acuta* recorded superior

performance with respect to callusing, registering high cllus index values. Successful regeneration of roots and shoots was obtained from leaf and stem calli of the experimental species. Half MS medium, supplemented with NAA and kinetin each at 1.0 mg/l, was standardised as the production medium, which recorded positive response in leaf calli of *S. cordifolia*, with respect to synthesis of ephedrine in chromatographic tests. Butanol : glacial acetic acid : water at 4:1:1 was identified as the appropriate solvent system with ninhydrine as the localizing spray. Incorporation of yeast extrat 2.0 g/l and the precursor, phenyl alanine 50.0-100.0 mg/l elicited synthesis of ephedrine in leaf and stem calli of *S. cordifolia*. Addition of the osmoregulant polyethylene glycol 2.0 per cent had a favourable effect on synthesis of ephedrine. Presence of ephedrine in *in vitro* cultures of *S. cordifolia* was confirmed by eliciting the cultures with autoclaved mycelia of *Pythium aphanidermatum* at 500.0 mg l^{-1}, 2.0 g l^{-1} and 5.0 g l^{-1}. Supplementing elicitation with precursor feeding was particularly beneficial to the synthesis of ephedrine. Apart from *S. cordifolia*, leaf callus cultures of *S. rhonlifolia* ssp. *rhonlifolia* synthesized ephedrine. Success in establishment of hairy root cultures depended on the efficiency of the strain of *Agrobacterium rhizogenes* employed. Strain A4 induced hairy roots in 50 per cent cultures and in leaf and stem calli of *S. cordifolia*. Successful liquid suspensions of experimental species could be established. *S. cordifolia* was the most effective with respect to proliferation in liquid suspensions. Elicitation, coupled with precursor feeding, produced the highest content of ephedrine in *in vitro* cultures. Leaf extracts of *S. cordifolia* recorded comparatively higher content of ephedrine.

Gymnema sylvestre

Internode, node, petiole, leaf lamina and root segments of Gymnema were evaluated as explants to initiate calli and found that internode and petiole were found to have maximum potential to initiate and proliferate calli. MS medium supplemented with 2,4-D (2 mg l^{-1}) and BA (1 mg l^{-1}) was selected as the basal medium due to high callusing and high saponin yields. Addition of stress inducing chemicals like mannitol, activated charcoal, peptone and malt extract enhanced saponin yields. The highest saponin yield per day per tube was produced from the medium comprising of MS + 2,4-D (2 mg l^{-1}) + BA (1 mg l^{-1}) + malt extract 1%. In cell suspensions the production of saponins was highest in the medium containing MS + 2,4-D (2 mg l^{-1}) + BA 1 mg l^{-1} + phloroglucinol (50 mg l^{-1}). Both calli and cell suspensions produced new groups of saponins which were not present in the plant extracts, suggesting that de novo synthesis occurred in the *in vitro* cultures.

Coscinium fenestratum

Leaf segments and leaf segments attached with petiole base produced compact yellowish coloured calli in coscinium. Callus index was highest with the hormonal combination IAA 1 mg l^{-1} + BA 1 mg l^{-1}. There was no regeneration from callus in any of the treatments tried. The alkaloid berberine was present in calli produced from leaf explants in solid ½ MS media in different treatment such as

BA 0.25 mg l^{-1}, 0.5 mg l^{-1}, BA 1 mg l^{-1} + IAA 2 mg l^{-1} and IAA 2 mg l^{-1} + 2,4-D 1 mg l^{-1}. The highest amount of berberine (0.150 µg/g callus) obtained among the different treatments tried in solid ½ MS medium was with IAA 2 mg l^{-1} and BA 1 mg l^{-1}. Considering the different treatment in ½ MS liquid medium, the berberine recovery was found to be highest when the phosphate ion source was reduced to 25% in the ½ MS liquid medium. The recovery was 10.079 µg/g callus. The *in vitro* derived callus had higher amounts of berberine than the *ex vitro* samples.

Tinospora cordifolia

Leaf, petiole and stem derived callus cultures of Vellanikkara and Madurai ecotypes of *Tinospora* were established *in vitro* MS media of full strength supplemented with NAA and BA or NAA and kin each at 2 mg l^{-1} was standardized as the production medium which recorded maximum berberine synthesis. Substituting sucrose with lactose maintaining a proportion of 2:1 and reducing the phosphorus level in basal medium to half the original strength resulted in increased levels of berberine synthesis. Incorporation of autoclaved mycelia of *Pythium aphanidermatum* at 0.5, 1.0 and 1.5 g l^{-1} and immobilization of calli with sodium alginate - calcium chloride complex revealed a positive influence on synthesis of berberine. When compared to *ex vitro* samples, *in vitro* cultures yielded higher quantities of berberine. The highest berberine yield (23.176 µg/g of callus) was obtained from stem cultures maintained in solid MS media supplemented with NAA 2 mg l^{-1} + BA 2 mg l^{-1} and autoclaved mycelia of *P. aphanidermatum* at 0.5 g l^{-1}.

Neem

Callus production from internodal segments was achieved in MS medium supplemented with varying levels of auxins. Methanol: water (30:70) was found to be the best solvent system for elution of azadiractin. The highest amount of 3.51 and 6.71ig g^{-1} azadiractin was estimated at 18th and 30th day, respectively, in MS medium containing 1.0 mg l^{-1} IAA. IAA in general appeared to induce higher production of azadiractin, compared to Kn.

9. WOMEN EMPOWERMENT NETWORKING IN KERALA THROUGH SCIENCE AND TECHNOLOGY

The DBT project on women empowerment Networking in Kerala through science and technology was sanctioned in January 2004. The project aims at providing consultancy services to farm women and women entrepreneurs as well as popularization and dissemination of women friendly agrobiotechnologies through intensive training programmes and monthly agroclinics. Initiating the activities of the project a consultancy consortium for conducting the agroclinic at institutional level and field level was formed. Four field level and two farm level agroclinics were conducted. A monitoring and evaluation committee was constituted for assessing the progress of the project. With the objective of technical empowerment of unemployed farm women in women friendly agrobiotechnologies such as vermicomposting, tissue

culture and floriculture, target groups were identified. Three field level demonstration units - ABARD vermicomposting unit, Mannuthy, Vanitha Pushpakrishi Samrakshana Samiti, Nattika and Vanitha Pookrishi Vikasana Samiti, Perinjanam in the respective identified technologies (Vermi composting, tissue culture and floriculture) and infrastructural facilities are being set up. A bench mark survey about the socio-economic profile of the members in the demonstration units was completed.

10. EXTERNALLY AIDED RESEARCH PROGRAMMES IN PLANT BIOTECHNOLOGY

The Departments of Plant Biotechnology at Vellanikkara and Vellayani and the Plant Tissue culture Laboratory at the RARS Kayamkulam could attract reasonable external funding for research (Annexure II). The total funding so far amounts to Rs. 650 lakhs. The agencies funding various biotechnology programmes include the Department of Biotechnology, Indian Council of Agricultural Research, Kerala State Council for Science, Technology and Environment, BRNS, US-India Funds, Cadbury Ltd. etc.

11. COLLABORATIVE RESEARCH PROGRAMMES

The SPIC Research Foundation, Chennai, the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram and the Indian Institute of Spices Research, Kozhikkod, have entered into collaborative research programmes in biotechnology with the KAU. The SPIC was co-operating in developing disease resistance in pepper using molecular techniques. The RGCB is interested in imparting gall midge resistance in rice through genetic transformation as well as developing disease resistant ginger varieties. It will be advantageous to have more collaborative programmes in biotechnology in areas of interest to the Kerala Agricultural University.

13. FACILITIES

A facility for tissue culture research was established at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara in 1981. The present Department of Plant Biotechnology consists of several culture rooms, media preparation rooms, inoculation rooms and hardening units, for *in vitro* propagation, *in vitro* crop improvement and *in vitro* secondary metabolite production; facilities to carry out molecular biology work, including genetic transformation. Most of the facilities were established through funding from external agencies. Research, teaching and training programmes in biotechnology and molecular biology are being carried out.

At the College of Agriculture, Vellayani, a Plant Tissue Culture Laboratory was established at the Department of Horticulture, in 1988 with the financial support from the US-India Funds. The present Department of Plant Biotechnology consists of several culture rooms, media preparation rooms, inoculation rooms and hardening units, for *in vitro* propagation, *in vitro* crop improvement and *in vitro* secondary metabolite production; facilities to carry out molecular biology work, including genetic transformation. Research, teaching and training programmes in biotechnology and molecular biology are being carried out.

At the Regional Agricultural Research Station, Ambalavayal, a Tissue Culture Propagation Unit was established in 1994. The unit has culture room, media preparation room, inoculation room and hardening units, for large scale *in vitro* propagation.

At the KHDP Centre, Vellanikkara a Plant Tissue Culture Laboratory was established in 1994 with the financial support from the Europian Economic Community. The laboratory has culture rooms, media preparation rooms, inoculation rooms and hardening units, for *in vitro* propagation and *in vitro* crop improvement.

At the Regional Agricultural Research Station, Kayamkulam a Plant Tissue Culture Laboratory was established in 1996 with the support from externally aided research project. The laboratory has culture room, media preparation room, inoculation room and hardening unit, for *in vitro* propagation and *in vitro* crop improvement.

At the Regional Agricultural Research Stations at Kumarakom and Pattambi and at the College of Agriculture, Padannakkad Plant Tissue Culture Laboratories were established. Large scale multiplication of banana and vanilla are being undertaken.

The list of major equipment available at the various centers are presented in Annexure IV.

13. MANPOWER

Currently there are thirty seven scientists associated with biotechnology and molecular biology research at the KAU (Annexure III). The Project Coordination Group of Plant Biotechnology consists of nineteen members and a Project Coordinator. Fifteen scientists have been trained in national and international institutions. Four scientists were awarded the Biotechnology National Associateship. One scientist was awarded the Commonwealth Post Doctoral Fellowship. One scientist was selected under the FAO overseas training programme. Fourteen scientists are working as faculty members in the Departments of Biotechnology at Vellanikkara and Vellayani. Four scientists are in the team of the CPBMB, Vellanikkara that received the "Biotech Product & Process Development & Commercialisation Award 2003" of the Department of Biotechnology, Ministry of Science and Technology, Government of India. Several scientists are serving as external examiners, selection committee members and question paper setters for the biotechnology programmes of nearby institutions and Universities.

14. ACADEMIC PROGRAMMES

Several post graduate students have been engaged in biotechnology research at the Kerala Agricultural University (Annexure I). Two post graduate students, (incidentally, faculty members of the KAU) *viz.*, Dr. P.A. Valsala, Associate Professor and Dr. M.R. Shylaja, Assistant Professor, Department of Plantation Crops and Spices, and one post graduate student of the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, bagged the prestigious Jawaharlal Nehru Award, for their Ph.D. thesis on *in vitro* pollination and fertilisation in ginger, exploitation of somaclonal variation in pepper and *in vitro* propagation of anthuriums, respectively.

During the recent restructuring of the syllabi, four courses in biotechnology (two theory courses and two work experience courses) have been included in the B.Sc. (Ag.) syllabus.

M.Sc. (Ag.) Plant Biotechnology programme was initiated at the University in 2003. Ten students per batch were admitted to the Department of Plant Biotechnology at Vellanikkara (six) and the Department of Plant Biotechnology at Vellayani (four). The two Departments consist of seven faculty members each.

15. TRAINING

Training programmes, are regularly offered to the unemployed youth, officers of the Department of Agriculture, officers of various development agencies, teachers etc. at the College of Agriculture, Vellayani and College of Horticulture, Vellanikkara. In addition, there used to be several visitors (including students, farmers, extension personnel and officials) to the biotechnololgy centres at Vellayani, Vellanikkara, Ambalavayal and Kayamkulam.

16. EXTENSION PROGRAMMES

The scientists of the biotechnology centres have been actively engaged in various extension programmes. They have published popular articles in leading publications and dailies and delivered talks and interviews *via* the All India Radio. They have been delivering talks in public functions organised by various agencies like Local bodies, Karshaka Sangham, Karshaka Congress, voluntary organisations and Science and Technology Entrepreneurship Development Agency. They have been handling classes in the various training programmes in tissue culture conducted by the KAU and other agencies. Television programmes about biotechnology have also been arranged. Books on Tissue Culture and Biotechnology were also published.

a) Training activities conducted by the Department

For teachers & Research Workers of the same discipline

a. Access to Biotechnology Information
b. Sequence Analysis of Nucleic acids & Proteins
c. Information Technology for Biological Sciences
d. Techniques in Plant Molecular Biology

(i) Extension workers in the same discipline

Staff of the Centre handle sessions on micropropagation and its commercialization in economic crops, genetic transformation and its application, applications of biotechnology in agriculture etc. in training programmes organised by the State Department of Agriculture, Commodity Boards and Nationalised Banks for the benefit of Agricultural Assistants, Technical Assistants, Field Officers, Agricultural Officers, Assistant Directors, Deputy Directors etc.

(ii) Other training programmes

a. Long-term training in Plant Tissue Culture and its applications offered for the benefit of entrepreneurs. This training programme has played a key role in moulding several tissue culture units in the State. One such unit is at present running under women entrepreneurship.

b. Training on plant tissue culture to the unemployed youth registered under Small Industries Service Institute is conducted as part of empowerment to unemployed youth for initiating sustainable units.

c. Transfer of Technology to farmers on management of tissue culture derived black pepper plants. This includes hardening, application of biofertilizer and biocontrol agents, organic farming etc. This is a sponsored programme of the Department of Biotechnology, Govt. of India, as a part of commercialization of tissue culture technology.

b) On farm evaluation of tissue culture derived plants

The protocol developed at CPBMP, College of Horticulture is being utilized at four Centres of KAU for rapid multiplication of identified varieties of black pepper. The centres include TC lab of RARS, Ambalavayal and Department of Plant Biotechnology, College of Agriculture, Vellayani.

Two thousand seven hundred and fifty tissue culture derived pepper plants have been hardened for on farm evaluation. One thousand nine hundred and fifty plants were distributed to 32 units. Both the tissue culture plants and conventional propagules were fortified with the biocontrol agent *Trichoderma* spp. The performance of tissue culture derived black pepper plants established earlier in farmer's field and KAU campuses were evaluated for their growth and yield. The growth, flowering, fruit set and yield of tissue culture derived plants were superior to the conventional propagules. RAPD profiling of 20 randomly selected tissue culture plant of Subhkara and Panchami, was done using 6 Operon primers each and the profiles indicated genetic uniformity among the micropropagated plants.

17. TECHNOLOGY TRANSFER AND CONSULTANCY

Protocols for the *in vitro* propagation of Red Banana, 'Nendran', pineapple, orchid and anthurium were transferred to Mitraniketan, a voluntary organisation at Vellanad, Thiruvananthapuram. Five thousand rupees per protocol were charged by the KAU. In addition, the organisation will have to pay ten per cent of the gross income through selling the plantlets, as royalty. Mr. C.M. Gopi, a progressive farmer and winner of the "Karshakasree" award has approached the KAU for making available protocol for the *in vitro* propagation of his banana variety "Quintal banana". Two scientists are actively involved in the functioning of the Biotechnology Centre of the State Department of Agriculture at Kazhakkoottam.

18. BIOTECHNOLOGY PROGRAMMES OF THE KAU AND THE REQUIREMENT OF THE STATE

Several aspects of biotechnology are being focussed at the Kerala Agricultural University.

In vitro propagation is one of the most widely exploited aspect of plant biotechnology, catering to the demand of farmers for quality planting materials. *In vitro* clonal propagation has great relevance in Kerala, where the production and productivity of most of the crops are below satisfactory levels.

In vitro techniques have been standardised for a number of crops, having commercial importance. However, the protocols evolved have to be refined, based on the feed-backs from farmers. Effective interaction among the scientists, extension personnel and the farmers is essential for popularising *in vitro* propagation in Kerala. Under the **Biotech Kerala Project** of the Government of Kerala, finalized with the help of Prof. V. L. Chopra, the Kerala Agricultural University has taken up the large scale tissue culture propagation of banana and vanilla, during the first phase. Disease free quality planting materials will be distributed. Two nucleus centers (Departments of Plant Biotechnology at Vellanikkara and Vellayani) and five production centers (RARS at Kayamkulam, Kumarakom, Pattambi and Ambalavayal and College of Agriculture, Padannakkad) are involved.

Molecular biology and genetic engineering tools are adopted at the Kerala Agricultural University for imparting resistance to various crop diseases, pests and environmental stress like drought and salinity. Molecular characterization of ecotypes and varieties of crops and medicinal plants, conservation of biodiversity, isolation and characterization of useful genes etc. are being attempted. *In vitro* production of secondary plant metabolites is another thrust area for research.

19. Award

The Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara bagged the prestigious **Biotech Product & Process Development & Commercialisation Award** of the Department of Biotechnology, Ministry of Science and Technology, Government of India, for the year **2003.**

Research activities at the Centre

Training and Technology Transfer from the Centre

BIOTECHNOLOGY RESEARCH AT KAU – ACHIEVEMENTS

Plant Tissue Culture

Piper nigrum

Zingiber officinale

Vanilla planifolia

Holostemma sp.

Hardening of Tissue Culture Regenerants

Piper nigrum

Vanilla planifolia

Field Evaluation of Tissue Culture Regenerants

Vanilla planifolia

Plant - Microbe Interaction

Endophytic nitrogen fixing bacteria in black pepper

Response of *P. nigrum and P. colubrinum* to *Phytophthora capsici*

Genetic Transformation

Callus induction in capsicum

BIOTECHNOLOGY RESEARCH AT KAU – ACHIEVEMENTS

Annexure I

LIST OF RESEARCH PROJECTS IN BIOTECHNOLOGY AT THE KERALA AGRICULTURAL UNIVERSITY

Project (01): *In vitro* propagation of plants of economic importance

Sub-project (01): Standardisation of *in vitro* propagation techniques

1 Standardisation of tissue / meristem culture techniques in important horticultural crops

2. Standardisation of tissue culture techniques in cardamom

3. Micro and macro propagation techniques in bougainvillea

4. Micro and macro propagation techniques in Indian gooseberry

5. *In vitro* propagation of black pepper

6. Collection, evaluation, cataloguing and genetic improvement to tailor better commercial varieties with export potential in *Anthurium* spp.

7. Tissue culture research on humid tropical plantation crops and spices

8. *In vitro* propagation and improvement of orchids

9. Field evaluation of tissue culture derived pepper plants

10. *In vitro* studies on *Gymnema sylvestre*, an anti diabetic plant

11. Rapid multiplication of rare and endangered medicinal plants

12. Rapid multiplication of cashew through *in vitro* methods

13. Triploid production in watermelon and anona through *in vitro* endosperm culture

14. Genetic fingerprinting in black pepper varieties and related species

15. Breeding for interspecific and intergeneric hybrids of orchids for commercial cultivation

16. Standardisation of tissue / apical meristem culture techniques in horticultural crops of Kerala
(BT-01-01-01/88 ACV 5 USDA/FERRO)

17. Relative response of explant material of *Anacardium occidentale* L. *in vitro*
(BT-01-01-02/88 ACV 5 KAU PG)

18. Relative response of explant material of *Myristica fragrans* Houtt. To *in vitro* culture
(BT-01-01-03/88 ACV 5 KAU PG)

19. Tissue culture on cocoa
(BT-01-01-04/88-VKA 16-Cad India Ltd.)

20. Somatic embryogenesis in cocoa (*Theobroma cacao*)
(BT-01-01-05/90 VKA 16 KAU PG)

21. *In vitro* clonal multiplication of cocoa
(BT-01-01-06/89 VKA 16 CAD India)

22. Mass multiplication through *in vitro* culture and callus mutagenesis in clove (BT-01-01-07/90 VKA 16-KAU)
23. *In vitro* propagation techniques in cinnamon (*Cinnamomum verum*) (BT-01-01-08/90-VKA 16-KAU)
24. Improvement of black pepper through *in vitro* techniques (BT-01-01-09/89 KAU NP)
25. Standardisation of *in vitro* techniques for rapid multiplication of difficult to root and endangered species of medicinal plants (BT-01-01-10/89 VKA 16 KAU NP)
26. Standardisation of *in vitro* propagation techniques in ginger (BT-01-01-12/90 VKA 16 KAU NP)
27. Standardisation of *in vitro* propagation techniques in banana (BT-01-01-13/88 VKA 15 KAU PG)
28. Standardisation of rapid clonal propagation through *in vitro* culture in pineapple (BT-01-01-14/89 VKA 15 KAU PG)
29. *In vitro* propagation of orchids (BT-01-01-15/89-VKA 15-ICAR-Co-ord.)
30. Standardisation of explant for *in vitro* propagation of *Dendrobium* spp. (BT-01-01-16/89 VKA 15 KAU PG)
31. Improvement of propagation efficiency of *Anthurium* species *in vitro* (BT-01-01-17/89 ACV 10 KAU PG)
32. Response of black pepper (*Piper nigrum* L.) to cloning through *in vitro* technique (BT-01-01-18/91 VKA 16 KAU PG)
33. Enhancing *in vitro* response of explants from mature jack (*Artocarpus heterophyllus* L.) trees (BT-01-01-19/91 ACV 10 KAU PG)
34. Standardisation of *in vitro* propagation technique in gurmar (*Gymnema sylvestre*) (BT-01-01-20/92 VKA 16 KAU PG)
35. Standardisation of medium supplements for shoot proliferation in *Dendrobium* (BT-01-01-21/92 VKA 15 KAU PG)
36. Somaclonal variation in black pepper (*Piper nigrum* L.) (BT-01-01-22/92 VKA (16) KAU-PG)
37. Induction of genetic variability in *Musa* sp. var. Nendran by *in vitro* methods (BT-01-01-23/92 VKA 5 KAU PG)
38. *In vitro* propagation of bijasal (*Pterocarpus marsupium* Roxb.) through tissue culture (BT-01-01-24/92 VKA F KAU PG)
39. Standardisation of *in vitro* propagation technique in clove, *Syzygium aromaticum* (BT-01-01-25 / 92 VKA 16 KAU PG)

40. Standardisation of *in vitro* techniques for the rapid clonal propagation of mango (*Mangifera indica* L.)
(BT-01-01-26 / 92 ACV 10 KAU PG)

41. Response of gladiolus to rapid cloning through *in vitro* techniques
(BT-01-01-27 / 93 VKA 15 KAU PG)

42. Plantlet regeneration through somatic embryogenesis in *Theobroma cacao* L.
(BT-01-01-28 / 93 VKA 2 KAU PG)

43. Effect of physiological preconditioning of explants and explant sources of *Myristica fragrans* Houtt. to *in vitro* culture establishment and growth.
(BT-01-01-29 / 92 ACV 10 KAU PG)

44. Optimising *in vitro* somatic embryogenesis in polyembryonic mango (*Mangifera indica* L.) varieties.
(BT-01-01-30 / 93 ACV 10 KAU PG)

45. *In vitro* propagation of Malabar white pine (*Vateria indica* L.) through tissue culture
(BT-01-01-31/93 VKA F KAU PG)

46. Standardisation of *in vitro* techniques for mass multiplication of *Aranthera* and *Dendrobium*
(BT-01-01-32 / 93 ACV 10 KAU PG)

47. Micropropagation of tree spices of Kerala
(BT-01-01-33 / 93 ACV 10 KAU DBT)

48. *In vitro* techniques for the rapid multiplication of *Kaempferia galanga* L
(BT-01-01-34 / 94 VKA 16 KAU)

49. Standardization of *in vitro* techniques for rapid multiplication of *Holostemma annulare* K. Schum.
(BT-01-01-34 (b)/ 94 VKA 16 KAU PG)

50. Clonal propagation of selected plus trees of Indian rosewood (*Dalbergia latifolia* Roxb.) through tissue culture
(BT-01-01-35 / 94 VKA (F) KAU PG)

51. Standardization of *in vitro* techniques for rooting, hardening and micrografting in cocoa (*Theobroma cacao* L.)
(BT-01-00-36 / 94 VKA 9 KAU PG)

52. Standardization of propagation techniques in Schefflera (*Schefflera arboricola* Hayata
(BT-01-01-37 / 94 VKA 16 KAU PG)

53. Evolving techniques for *in vitro* somatic embryogenesis in polyembryonic mango (*Mangifera indica* L.) varieties
(BT-01-01-38/94 ACV-10-KAU PG)

54. Indirect organogenesis and embryogenesis in *Kaempferia galanga* L.
(BT-01-01-39/95 VKA-15-KAU PG)

55. Evolving techniques for *in vitro* somatic embryogenesis in monoembryonic mango (*Mangifera indica* L.) varieties
(BT-01-01-40/94 ACV-10-KAU PG)

56. Evolving techniques for the *in vitro* propagation of economically important mono and poly embryonic mango (*Mangifera indica* L.) varieties of Kerala
(BT-01-01-41/95 ACV 15 KAU)

57. Standardisation of *in vitro* techniques for rapid multiplication of *Trichopus zeylanicus* Gaetrtn.
(BT-01-01-42/95 VKA-16-KAU PG)

58. *In vitro* somatic organogenesis in coconut (*Cocos nucifera*)
(BT-01-01-43/95 VKA-16-KAU)

59. *In vitro* response and causes of recalcitrancy in coconut (*Cocos nucifera* L.)
(BT-01-01-44/95 VKA-16-KAU PG)

60. Production of triploid tamarind and garcinia through endosperm culture
(BT-01-01-45/95 VKA-16-KAU)

61. Improvement of *in vitro* somatic embryogenesis in cashew (*Anacardium occidentale* L.)
(BT-01-01-46/97 ACV-10-KAU PG)

62. *In vitro* cloning and improvement of philodendrone cv. Majesty
(BT-01-01-47/97 VKA-15-KAU PG)

63. *In vitro* propagation of *Ailanthus triphysa* (Dennst.)
(BT-01-01-48/97 VKA-20-KAU PG)

64. *In vitro* propagation of promising gladiolus (*Gladiolus* sp.) varieties
(BT-01-01-49/97 ACV-10-KAU)

65. *In vitro* endosperm culture in *Annona squamosa* L. and *Syzygium aromaticum* L.
(BT-01-01-50/97 VKA-16-KAU PG)

66. Overcoming developmental abnormalities of *in vitro* somatic embryos in mango (*Mangifera indica* L.) and cashew (*Anacardium occidentale* L)
(BT-01-01-51/98 ACV-10-KAU PG)

67. Molecular evaluation of genomic stability of banana plants developed by *in vitro* clonal propagation
(BT-01-01-52/99 ACV-15-KAU PG)

68. Standardisation of *in vitro* techniques for the rapid clonal propagation of bael (*Aegle marmelos* (L.) Corr.)
(BT-01-01-53/99 ACV-15-KAU PG)

69. Micropropagation of teak (*Tectona grandis* Linn.) through *in vitro* techniques
(BT-01-01-54/99 VKA-20-KAU PG)

70. Micropropagation and crop improvement of cordyline (*Cordyline terminalis*) (L.) Kunth.)
(BT-01-01-55/99 VKA-15-KAU PG)

71. *In vitro* multiplication and genetic improvement of tuberose (*Polianthes tuberose* Linn.)
(BT-01-01-56/99 VKA-15-KAU PG)

Sub-project (02): *Ex vitro* establishment of plantlets

72. *Ex vitro* establishment of jack (*Artocarpus heterophyllus*) plantlets
(BT-01-02-01/88 ACV 10-KAU PG)
73. Standardisation of media and containers for ex *vitro* establishment of anthurium plantlets produced by leaf culture
(BT-01-02-02/92 ACV 10-KAU PG)
74. Field evaluation of calli clones of black pepper (*Piper nigrum* L.)
(BT-01-02-03/95 VKA-16-KAU)
75. RAPD analysis to assess the genetic stability in tissue culture derived black pepper (*Piper nigrum* L.) plants
(BT-01-02-04/97 VKA-16-KAU PG)
76. Characterisation of field established tissue culture derived black pepper (*Piper nigrum* L.) plants using morphological, cytological and molecular markers
(BT-01-02-05/97 VKA-9-KAU PG)
77. Physiological aspects of ex *vitro* establishment of tissue culture derived orchid (*Dendrobium* spp. Var. Sonia-17) plantlets
(BT-01-02-06/98 ACV-22-KAU PG)

Sub-project (03): *In vitro* and *ex vitro* propagation techniques

78. *In vitro* multiplication and fertilization of hardening techniques in pineapple (*Ananas comosus* L. Merr.)
(BT-01-03-01/ 92 VKA 15 KAU PG)
79. Standardisation of *in vitro* and ex *vitro* propagation in *Anthurium andreanum*
(BT-01-03-02 / 92 ACV 10 KAU NP)
80. Rapid asexual multiplication of hybrid seedlings of pineapple
(BT-01-03-03 /92 KNR 10 KAU NP)
(BT-01-03-04 / 93 VKA 16 KAU PG)
** Transferred to BT 04-01-01)
81. Refinement of *in vitro* propagation techniques in pineapple variety Mauritius and mass multiplication of elite clones
(BT-01-03-05 / 94 VKA 15 KAU PG)
82. Micropropagation in selected varieties of *Anthurium andreanum* (Lind.)
(BT-01-03-06 / 94 ACV 10 KAU PG)
83. Micropropagation of Phalaenopsis
(BT-01-03-07 / 94 VKA 15 KAU PG)
84. Cost effective methods and devices for home scale adoption of plant tissue culture
(BT-01-03-08 / 94 ACV 10 KAU PG)

85. Low cost technology for propagation of *Musa* (AAB) 'Nendran'
(BT-01-03-09 / 94 VKA 15 KAU PG)

Project (02): Improvement of crop plants through genetic engineering

Sub project (01): Somatic cell culture studies

86. Tissue culture and somaclonal variation in ginger (*Zingiber officinale* Rox)
(BT-02-01-01/89-VKA-16-KAU)
87. Callus induction and plantlet regeneration in *Cucumis sativus* L. by anther culture
(BT-02-01-02/90-ACV 10-KAU/PG)
88. Anther culture in cocoa (*Theobroma cacao* L.)
(BT-02-01-03/91- VKA 2 KAU PG)

Sub project (02): *In vitro* mutagenesis, utilization of somaclonal variability

89. *In vitro* mutagenesis in ginger (*Zingiber officinale* Rose)
(BT-02-02-03(a)/89 VKA-16-KAU)
90. *In vitro* production of hybrids in ginger, *Zingiber officinale* Rox
(BT-02-02-03/91 VKA-16-KAU PG)
91. Protoplast isolation, culture and regeneration in mango (*Mangifera indica* L.)
(BT-02-02-04/98 ACV-10-KAU PG)
92. *Agrobacterium* mediated genetic transformation in black pepper (*Piper nigrum* L.)
(BT-02-02-05/98 VKA-9-KAU PG)
93. Parasexual fertilization of *Piper nigrum* L. and *Piper colubrinum* Link. through protoplast fusion
(BT-02-02-06/99 VKA-16-KAU PG)
94. Somatic embryogenesis in banana Musa (AAB) 'Nendran'
(BT-02-02-06/99 VKA-16-KAU PG)
95. Refinement of *in vivo* and *in vitro* pollination techniques in turmeric (*Curcuma domestica* Val.)
(BT-02-02-07/99 VKA-16-KAU PG)

Sub project (03): *In vitro* pollination and fertilization

96. *In vitro* pollination, embryo rescue and germination studies in ginger, *Zingiber officinale* Rox
(BT-01-00-37/94 VKA-16-KAU PG)
97. Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination
(BT-02-03-02/97 VKA-16-KAU PG)

Project (04): *In vitro* production of secondary metabolites

98. *In vivo* and *in vitro* screening of *Sida* spp for ephedrine content
(BT-04-01-01 / 93 VKA 16 KAU PG)
99. *In vitro* callus induction and its exploitation in *Coscinium fenestrum* (Gaertn.) Colebr
(BT-04-01-02 / 97 VKA 16 KAU PG)

100. *In vitro* callus induction in gurmar (*Gymnema sylvestre* R. Br.) for secondary metabolite synthesis
(BT-04-01-02 / 98 VKA 16 KAU PG)

101. Genetic transformation for hairy root induction in Adakodien (*Holostemma adakodien* K. Schum)
(BT-04-01-03/99 VKA-16-KAU PG)

Project (05): Evolving stress (biotic/abiotic) tolerant plants.

102. Exploiting somaclonal variation for evolving saline tolerant rice plants
(BT-05-00-01/95 ACV 9 KAU)

Sub project (02) Calli clones

103. Assessment of somaclonal variation in calli clones of black pepper (*Piper nigrum* L.)
(BT-05-02-01/95 VKA-16-KAU PG)

Sub project (03) Biotic stress tolerance

104 Exploitation of somaclonal variation for tolerance / resistance to soft rot and bacterial wilt in ginger (*Zingiber officinale* Rosc.)
(BT-05-03-01/95 VKA-16-KAU)

105. Variability analysis in calliclones of black pepper (*Piper nigrum* L.)
(BT-05-03-02/99 VKA-16-KAU PG)

106. Evaluation of pathogenesis related proteins in relation to *Phytophthora* foot rot in black pepper (*Piper nigrum* L.)
(BT-05-03-03/99 VKA-16-KAU PG)

Project (01) Foot rot of pepper

107. Genetic transformation of black pepper (*Piper nigrum* L.) for *Phytophthora* foot rot resistance / tolerance
BT/01-00-02-2001 VKA(16)SPIC-KAU/PG *Lisamma Joseph (2000.22.01) (P.A. Valsala)*

Project (02) Soft rot of ginger

108. *Agrobacterium* mediated genetic transformation of ginger (*Zingiber officinale* Rosc.)
BT/02-00-01-2000 VKA(16)KAU/PG *B. Suma (99.22.01) (R. Keshavachandran)*

Project (03) Biotic stress in rice

109. Molecular basis of resistance in rice to sheath blight disease incited by *Rhizoctonia solani* Kuhn
BT/03-00-01-2000 ACV(18)KAU*PI- Dr. Kamala Nayar*

Project (04) Root (wilt) of coconut

110. Detection of phytoplasma in root (wilt) affected coconut palms by molecular techniques
BT/04-00-01-2000 VKA(18)KAU *PI- Dr. D. Girija*

Project (05) Variability in vegetatively propagated crops

111. Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)
BT/08-00-02-2001VKA(16)KAU/ PG *G. Shankar (2000.12.19) (Alice Kurien)*

112. Morphological and molecular characterisation of variability in *in vitro* derived seedlings of vanilla (*Vanilla planifolia* Andrews)
BT/08-00-03-2002 VKA(10) DBT/PG *K. K. Hena (2001.12.14) (P.A.Nazeem)*

113. *Agrobacterium* mediated genetic transformation in *Dendrobium*
BT/08-00-04-2002 ACV(15)KAU/PG *R. Swarna Piria (2001.22.01) (K.Rajmohan)*

114. Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)
BT/08-00-05-2002 VKA(16)KAU /PG *Resmi Paul (2001.22.09) (Shyalaja)*

Project (06) *In vitro* propagation of crops

115. Studies on *in vitro* shoot tip culature of banana for the development of a cost effective small scale production system.
BT/06-00-04-2001 VKA(15)KAU/PG *N.Sundararasu (2000.12.16) (K.Aravindakshan)*

116. *In vitro* somatic embryogenesis in bael [*Aegle marmelos* (L.) Corr.] varieties
BT/06-00-05-2002 ACV(16)KAU/PG *Hima Sugathan (2001.12.06) (G.R.Sulekha)*

117. Rapid propagation and conservation of selected leguminous medicinal plants using *in vitro* techniques
BT/06-00-06-2002 ACV(16)KAU/PG *Deepa S. Nair (2001.22.04) (B.R.Reghunath)*

118. Micropropagation of *Dendrobium* hybrids
BT/06-00-07-2003 ACV(9)DBT/PG S. Sivamani *(2002.11.30) (Lekha Rani)*

Project (07) Molecular documentation of crop genetic resources

119. Morpho anatomical and molecular characterisation of Dendrobium Sw. cultivars
BT/07-00-01-2000 ACV(15)KAU/PG *N. Padmanabha Pillai (99.22.10) (Sabeena George)*

120. Molecular characterisation of *Ralstonia solanacearum* (Smith) Yabuuchi et al. causing bacterial wilt in solanaceous vegetables
BT/07-00-03-2000 VKA(5)KAU/PG *Deepa James (99.11.09) (D. Girija)*

121. Molecular characterisation *Piper* spp. using RAPD techniques
BT/07-00-04-2000 VKA(16)KAU/PG *C. Murugan (99.12.19) (Sujatha)*

122. Morphological, biochemical and molecular markers for the genetic analysis in cashew (*Anacardium occidentale* L.)
BT/07-00-04-2001 VKA(9)KAU/PG *D. Usha Vani (2000.11.33) (Jayalakshmi)*

123. Isozyme variation in *Areca catechu* L. and allied species
BT/07-00-05-2001 VKA(16)KAU/PG *A. Arul Swaminathan (2000.12.13) (T.U.George)*

124. Biochemical and molecular characterisation of 'Njavara' types of rice (*Oryza sativa* L.)
BT/07-00-06-2001 VKA(9)KAU/PG *Sanal Kumar, P. (2000.21.15) (Elsy)*

125. Random amplified polymorphic DNA (RAPD) analysis of banana (*Musa* spp.)
BT/07-00-07-2001 ACV(15)KAU/PG *C. Rajamanickam (2000.22.06) (K.Rajmohan)*

126. Genetic variability analysis in Indlan indigo (*Indigofera tinctoria* L.) using Random amplified polymorphic DNA (RAPD) technique
BT/07-00-08-2003 ACV(16)KAU/PG *M. Neema.(2002.12.10) (Jessykkutty)*

127. Characterisation of traditional mango (*Mangifera indica* L.) varieties of southern Kerala
BT/07-00-09-2003 ACV(15)KAU/PG *S. Simi.(2002.22.02) (K. Rajmohan)*

Project (09) *In vitro* production of secondary plant metabolites

128. Utilisation of *in vitro* cultures of chittamrithu (*Tinospora cordifolia* Miers.) for berberine
BT/09-00-01-2000 VKA(16)KAU/PG *M. Kalimuthu (99.12.17) (Asha Shankar)*

Project (10) Genetic characterisation and improvement of beneficial micro-organisms

129. Characterisation of *Ralstonia solanacearum* (Smith) Yabuuchi et *al.*, causing bacterial wilt in ginger using molecular marker
BT/10-00-01-2002 VKA(5)KAU/PG *P. K. Sambasivam (2001.11.53) (D.Girija)*

March 2004 onwards

Project (01): *In vitro* propagation of crop plants

130. Standardisation of protocol for *in vitro* fertilization and seed germination in Zingibaraceous crops – ginger, turmeric and kacholam
BT/01-00-01-2003 VKA(19)KAU (*Dr. P.A. Valasala)*

131. Large scale production of banana plantlets through *in vitro* methods turmeric and kacholam
BT/01-00-02-2003 VKA(15)KAU (*Dr. K. Aravindakshan)*

132. *In vitro* mutagenesis in rice (*Oryza sativa* L.)
BT/01-00-03-2004 VKA(9)KAU/PG *(R. Ambika Rajendran) (2003.11.33)*

133. *In vitro* propagation in two commercial diploid bananas of Kerala
BT/01-00-04-2004 VKA(15)KAU/PG *(C.P.Sapheera) (2003.12.04)*

Project (02): *In vitro* crop improvement

134. Induction of genetic variability in ginger *(Zingiber officinale Rosc.)* through in vitro fertilization.
BT/02-00-01-2004 VKA(19)KAU/PG *(A. Rathidevi) (2003.11.55)*

135. Induction of variability in *Vanilla planifolia* Andeus through intra / interspecific hybridization and embryoculture technique.
BT/02-00-02-2004 VKA(19)KAU/PG *(Blessy Paul) (2003.11.57)*

Project (05): Genetic modification of plants and microbes

136. Genetic transformation of chilli (*Capsicum annuum* L.) with osmotin gene
BT/05-00-01-2004/VKA (19) KAU/ PG (T. Resmy Henry) (2003-11-50)

137. Genetic transformation for hairy root induction and enhancement of secondary metabolites in Aswagandha (*Withania somnifera* (L.) Dunal)
BT/05-00-02-2004/VKA (19) KAU/PG (Smini Varghese 2003-11-53)

138. *Agrobacterium tumefaciens* mediated genetic transformation in Kudangal (*Centella asiatica* (L.) Urban)
BT/05-00-03-2004/ACV(19) /KAU/PG (V. Nanditha Krishnan) (2003-11-54)

139. *In vitro* regeneration and *Agrobacterium* mediated transformation in tomato (*Lycopersicum esculentum*
Mill) in relation to disease resistance against Groundnut bud necrosis virus
BT/05-00-04-2004/ACV (9)/KAU/PG (P. Ramjitha) (2003-11-58)

140. *Agrobacterium* mediated genetic transformation in Koduveli (*Plumbago* spp. (L))
BT/05-00-05-2004/ACV (16) /KAU / PG (Roshna Bhaskar) (2003-11-60)

Project (06): Molecular marker analysis

141. Variability of chakkarakolli (*Gymnema sylvestre* R. Br.) using morphological, biochemical and molecular markers.
BT/06-00-01-2004/VKA(19)/KAU/ PG (Smita Nair) (2003-11-49)

142. Rooting of microshoots and *ex vitro* establishment of plantlets of bael *(Aegle marmelos* (L.) Corr.)
BT/06-00-02-2004/ACV(16)/KAU/ PG (Surya D. Aniyan) (2003-12-13)

143. *In vitro* multiplication and DNA fingerprinting of selected hybrids and their parents in *Anthurium andreanum* Linden.
BT/06-00-03-2004/ACV(9)/KAU/ PG (K. Yasin Jeshima) (2003-21-22)

Project (07): Proteomics and genomics

144. Differentiated expression of genes involved in anthocyanin pigmentation in Red banana and green red clones
BT/07-00-01-2004/ACV(19)/KAU/ PG (Rehna Augustine) (2003-11-52)

145. Molecular characterization of 3 hydroxy 3 methyl glutaryl-CoA-reductase(hmgr) gene from solanaceous plants
BT/07-00-02-2004/VKA(19)/KAU/ PG (Smitha Jose)(2003-11-56)

146. Isolation and characterization of ß- 1,3 glucanase gene in *Piper* spp.
BT/07-00-03-2004/VKA(19)/KAU/ PG (Mable Rose George) (2003-11-59)

Annexure II

EXTERNALLY AIDED RESEARCH PROJECTS IN PLANT BIOTECHNOLOGY AT KAU

Externally aided research projects in plant biotechnology at Vellanikkara

Sl. No.	Project	Agency	Duration	Fund Rs. lakh
1	2	3	4	5
1	Network project on Improvement of selected spices through biotechnology tools	DBT	3 years	48.65
2	Women Empowerment Networking in Kerala through Science & Technology	DBT	3 years	26.74
3	On farm evaluation of tissue culture derived black pepper plants	DBT	3 years	38.70
4	Genetic transformation for biotic stress tolerance in bell pepper	DBT	3 years	22.56
5	Genomic library construction and cloning of genes for PR proteins in Black pepper	STEC	3 years	5.61
6	Micropropagation and development of seedless Malabar Tamarind through *in vitro* techniques	ICAR	3 years	8.67
7	Induction of variation through tissue culture techniques and evaluation of the variants for phytophthora foot rot tolerance/resistance in black pepper	Govt. of India	3 years	7.41
8	Establishment of *in vitro* gene bank in select medicinal species	DBT	3 years	3.45
9	Biotechnological interventions and opportunities toward enchansing crop production in Kerala	Biotech Kerala	2 years	10.00
10	Studies on genetic diversity of teak using AFLP markers	DBT	3 years	12.85
11	Tissue culture research in cashew	DBT	5 years	20.00
12	Distributed information system	DBT	5 years	1 crore
13	Induction of variability in Zingiberaceous crops (ginger, turmeric and kacholam)	DBT	3 years	20.4
14	Characterization and classification of banana varieties of Kerala using molecular markers	STEC	3 years	4.66
15	Tissue culture on cocoa	Cadbury	5 years	7.00
16	*In vitro* propagation of black pepper	DBT	5 years	9.00
17	Pilot Project on Tissue Culture	GOK	3 years	10.00
18	Field evaluation of tissue culture derived black pepper plants	DBT	3 years	15.00
19	*In vitro* studies on *Gymnema sylvestre*, an anti diabetic plant	DBT	3 years	14.00

1	2	3	4	5
20	Rapid multiplication of rare and endangered medicinal plants	ICAR	3 years	9.00
21	Triploid production in watermelon and anona through *in vitro* endosperm culture	DBT	3 years	10.00
22	Tissue culture research on humid tropical plantation crops and spices	DBT	5 years	70.00
23	Rapid multiplication of cashew through *in vitro* methods	ICAR	5 years	8.00
24	Hybridisation in ginger through *in vitro* pollination	ICAR	3 years	7.00
25	Micropropagation of *Ailanthus triphysa* and *Pterocarpus marsupium*	ICAR	3 years	10.00
26	Micropropagation of Bejasal (*Pterocarpus marsupium)*	ICAR	3 years	8.00
				506.7

Externally aided research projects in plant biotechnology at Vellayani

Sl. No.	Project	Agency	Duration	Fund Rs. lakh
1	Characterisation of traditional mango varieties of southern Kerala	ICAR	3 years	17.00
2	Biotechnological interventions and opportunities toward enchansing crop production in Kerala	GOK	2 years	40.00
3	Crop improvement in orchids *via in vitro* mutagenesis	BRNS	3 years	7.00
4	Molecular and physiological characterization of banana varieties	KSCSTE	3 years	10.00
5	Breeding for interspecific and intergeneric hybrids of orchids for commercial cultivation	DBT	3 years	14.00
6	*In vitro* propagation and improvement of orchids	DBT	5 years	14.00
7	Evolving techniques for the *in vitro* propagation of economically important mono and poly embryonic mango varieties of Kerala	ICAR	5 years	7.00
8	Pilot Project on Tissue Culture	GOK	3 years	10.00
9	Micropropagation of tree spices of Kerala	DBT	3 years	6.00
10	Standardisation of tissue/meristem culture techniques for the *in vitro* propagation of important horticultural crops of Kerala	US-India Funds	5 years	18.00
				143.00

Annexure III

SCIENTISTS ASSOCIATED WITH BIOTECHNOLOGY RESEARCH AT KAU

Sl. No.	Name and designation	Tissue culture	Mole-cular biology	Training in India	Training abroad	Student project/ KAU project	Externally aided project (PI)
1	2	3	4	5	6	7	8
College of Agriculture, Vellayani							
1	K. Rajmohan Associate Professor	✓	✓	✓		✓	✓
2.	K. B. Soni Assistant Professor	✓	✓	✓		✓	✓
3	B. R. Reghunath Associate Professor	✓		✓	✓	✓	✓
4	D. Wilson Associate Professor	✓				✓	
5.	Uma Maheswaran Assistant Professor		✓	✓		✓	✓
6.	Anith, Assistant Professor		✓	✓	✓	✓	v
7.	G. R. Sulekha Associate Professor	✓				✓	
8.	P. Sivaprasad Associate Professor			✓		✓	✓
9.	M.M. Viji Assistant Professor		✓	✓		✓	
10.	Hebsy Bai Associate Professor	✓				✓	✓
11.	Krishna Prasad Assistant Professor		✓	✓	✓		✓
12.	A. Naseema Associate Professor		✓	✓		✓	✓
13.	Sheela, V.L.	✓		✓		✓	✓
14.	Jayalekshmi, Assistant Professor		✓	✓		✓	
College of Horticulture, Vellanikkara							
15.	P.A. Nazeem Associate Professor	✓	✓	✓	✓	✓	✓
16.	R. Kesavachandran Associate Professor	✓	✓	✓	✓	✓	✓
17.	Augustine Associate Professor		✓	✓		✓	
18.	P. C. Rajendran Associate Professor	✓		✓		✓	✓

1	2	3	4	5	6	7	8
19.	D. Girija Assistant Professor	✓	✓	✓		✓	✓
20.	P.A. Valsala Associate Professor	✓		✓		✓	✓
21	Lizamma Joseph Assistant Professor	✓	✓	✓		✓	✓
22.	Achamma Oommen Associate Professor	✓				✓	
23.	M. R. Shylaja Assistant Professor	✓		✓		✓	✓
24.	P.K. Rajeevan Associate Professor	✓		✓		✓	✓
25.	P.K. Valsalakumari Associate Professor	✓		✓		✓	✓
26.	K. Aravindakshan Associate Professor	✓		✓		✓	✓
27.	Babylatha Associate Professor	✓				✓	
28.	Ashashanker Assistant Professor	✓		✓		✓	✓
29.	V.S. Sujatha Associate Professor	✓	✓	✓		✓	✓
30.	C.R. Elsy Assistant Professor		✓	✓	✓		✓
31.	C. T. Abraham Associate Professor	✓		✓	✓	✓	
College of Forestry, Vellanikkara							
32.	N.K. Vijayakumar Professor	✓	✓	✓		✓	
Pineapple Research Station, Vellanikkara							
33.	Sudhadevi Associate Professor	✓				✓	
College of Agriculture, Padannakkad							
34.	Swapna Alex Assistant Professor		✓	✓			
35.	Pradeep Kumar Assistant Professor	✓	✓	✓		✓	✓
RARS, Kayamkulam							
36.	Shyam S. Kurup Professor	✓		✓		✓	✓
RARS, Kumarakom							
37	Sajan Kurien Associate Professor	✓		✓			✓

Annexure IV

EQUIPMENT / FACILITIES AT THE DIFFERENT BIOTECHNOLOGY CENTRES OF KAU

Sl No		COH Vellani-kkara	COA Vella-yani	RARS Kayam-kulam	RARS Ambala-vayal	RARS Patta-mbi	RARS Kuma-rakom	COA Padan-nakad
1	PCR machine	✓	✓	✓				
2	Refrigerated microcentrifuge	✓	✓	✓				
3	High speed centrifuge	✓						
4	Electrophoresis unit	✓	✓	✓				
5	Gel documentation system	✓	✓	✓				
6	Biolistic DNA delivery system	✓						
7	Hybridisation incubator	✓	✓					
8	Water purification system	✓						
9	U V Visible spectrophotometer	✓	✓	✓				
10	Elisa reader		✓					
11	Circulatory waterbath	✓						
12	Incubator shaker	✓						
13	Indubator	✓	✓					
14	Gel drier	✓						
15	Electroblotter	✓	✓					
16	Zoom stereo microscopes	✓	✓					
17	Binocular microscopes	✓	✓	✓	✓	✓	✓	✓
18	Electronic balances	✓	✓	✓	✓	✓	✓	
19	Gyratory shakers		✓	✓	✓	✓		
20	Micro pipettes	✓	✓	✓	✓			✓
21	Autoclaves	✓	✓	✓	✓	✓	✓	✓
22	Double glass distillation units	✓	✓	✓	✓	✓	✓	✓
23	Laminar air flow chambers	✓	✓	✓	✓	✓	✓	✓
24	Culture racks	✓	✓	✓	✓	✓	✓	✓
25	Ovens	✓	✓	✓	✓	✓	✓	✓
26	Deep freezer	✓	✓					
27	Ice flaking machine	✓	✓					

Annexure V

PUBLICATIONS OF KAU SCIENTISTS IN BIOTECHNOLOGY

Achuthan, C. R., Babu, T. D., Jasmine, P. S and Nazeem, P. A. 2000. Expression of type-2 PR protein ß -1,3 glucanase in black pepper (*Piper nigrum* L.) infected with *Phytophthora capsici. Indian Science Congress, Pune, India* p 38

Achuthan. C.R., Nazeem. P.A., Babu.T.D. ,Girija, D. and Keshavachandran, R. 2002. Expression of ß 1,3 glucanase in relation to *Phytophthora* foot rot disease of *Piper nigrum*. Paper presented in *International Symposium on Molecular Approached for improved Crop Productivity and Quality*, CPMB, TNAU, Coimbatore.

Alex, S. and Pannikkar, B. 2000. Cadheins as prognostic markers in hydatidiform moles. *Proc. of Twelfth Kerala Sci. Congr.* p 319-321

Alex, S. Balaram, P. and Pannikkar, B. 1999. Immobilisation detection of tumor suppressor genes in complete hudatidiform moles (CHM). *Proc. of XV Asia Pacific Cancer Conference.* p 209

Alex, S., Pannikkar, B, and John, M. 2000. C-erbB2- Ras pathway in Gestational Trophoblastic Disease. *Proc. of 17th Annual convention of Indian Assoc. of Cancer Research.* p 138

Alex, S. and Pannikkar, B. 1999. Is NM23 a regulatory protein in the cell proliferation pathway. *Proc. of Eleventh Kerala Sci. Congress.* p 179-182

Anaz, M. A. and Vijayakumar, N. K. 1996. Micropropagation of neem (*Azadirachta indica*) and acacia (*Acacia auriculiformis*). *Myforest* 32: 283-288

Ancy, T.K., Kurien, S., Augustin, A. and Balachandran, P.V. 1998. Urease activity in banana fruit. *Journal of Plant Nutrition* 21: 2127-2140

Anita, S., Priya, L., Rajmohan K. and Swapna Alex. 2000. Comparison of chlorophyll content, water loss and anatomical features of leaves of the normal, *in vitro* cultured and hardened *Dendrobium* hybrid plants. *J. Orchid Soc. India* 14: 41-46

Anu, G. K., Geetha, C.K. and Valsalakumari, P.K. 2003. Rapid multiplication of tuberose (*Polianthus tuberosa* Linn.) var. Single from scale - stem sections - *Abstracts:* National Symposium on Recent Advances in Indian Floriculture. *ISOH,* IARI, New Delhi. p. 56.

Anu G. Krishnan, Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 2003. Callus mediated organogenesis in tuberose cv. Single from inflorescence segments. *Abstracts:* National Symposium on Recent Advances in Indian Floriculture. *ISOH,* IARI, New Delhi.

Aravindakshan, K. and Shanmugavelu, K. G. 1989. Studies on *in vitro* propagation of diploid banana hybrids (AA) through shoot tip culture. *Proc. Nat. Sem. Molecular Biology and Biotechnology,* 11-13 January 1989, Kottyam.

Aravindakshan, K. and and Shanmugavelu, K. G. 1990. Effect of certain hardening techniques on growth of *in vitro* produced plants of banana cv. Anaikamban (AA). *Extended Abst. Nat. Sem. Biotechnological Approaches for Upgradation of Agriculutural and Horticultural Crops*, September 1989, Coimbatore.p. 5-6

Aravindakshan, K. and and Shanmugavelu, K. G. 1990. Studies on *in vitro* clonal multiplication of diploid banana hybrids (AA) through shoot tip culture. *Extended Abst. Nat. Sem. Biotechnological Approaches for Upgradation of Agriculutural and Horticultural Crops*, September 1989, Coimbatore.p. 3-4..

Augustin, A., Anilkumar, A. S, Raj, M. and Gopal, K. S. 1998. Biochemical screening of *Kaempferia galanga* (Kacholam). *Internat.Conference on Medicinal Plants*, Bangalore, 16 to 19 February, 1998.

Babu, T. D., Achuthan, C. R., Nazeem, P. A. and Nair, R. V. 2000. Genomic DNA isolation and RAPD analysis in the tree spice- Nutmeg (*Myristica fragrans) Indian Science Congress, Pune, India*

Babu.T.D.,Nazeem.P.A., Keshavachandran. R., Achuthan.C.R., Girija.D and Peter, K.V. 2003. Detection of genetic diversity in *Piper* species using RAPD and AFLP markers. Paper presented in *National Seminar on New Perspectives in Spices, Medicinal and Aromatic Plants*, ISS, Goa. p 1-8

Baby Lissy Markose, Abdul Wahab, M., Augustin, A. and Rajan, S. 2000.Peroxidase activity in the defence mechanism of bacterial wilt in chilli (*Capsicum Annuum*.L.). *Proceedings of the Twelfth Kerala Science Congress* 477-479

Baby Lissy Markose, Augustin, A., Peter, K.V and Rajan, S. 2001. Effect of protein on the bacterial wilt resistance in chilli (*Capsicum annum* L.). *Proceedings of the Thirteenth Kerala Science Congress* January 2001,Trissur,325-327.

Bhaskar, J., Aravindakshan, M., Valsalakumari, P.K. and Rajeevan, P.K. 1993. Micropropagation studies in banana. *South Indian Hort. 41: 186-191*

Bhaskar, J., Aravindakshan, M., Rajeevan P.K and Valsalakumari, P.K 1992. *In vitro* propagation of banana. Paper presented at the *Golden Jubilee symposium on optimization of productivity in banana*, Pune.

Bhaskar, J., Aravindakshan, M., Rajeevan, P.K. and Valsalakumari, P.K 1992. Micropropagation of banana clones. *Banana Newsletter* 15: 6

Bhaskar, J. and Rajeevan, P.K. 2002. Embryo culture of vanda 'John Club'. *Proceedings of the 14th Kerala Science Congress*, STEC, Trivandrum, 29-31st January, 2002, Kochi.

Bhaskar, J. and Rajeevan, P.K. 2003. Controlling phenolic blackening in *in vitro* cultures of Phalaenopsis. *Abstracts:* National Symposium on Recent Advances in Indian Floriculture. *ISOH*, IARI, New Delhi. p 59

Bhaskar, J. and Rajeevan, P.K. 2003. Role of activated charcoal and triadimefon on *in vitro* rooting of *Phalaenopsis* var. Diana Pinky. *One hundred Research Papers in Floriculture:* National Symposium on Recent Advances in Indian Floriculture. *ISOH*, IARI, New Delhi. p 198-200

Bhaskar, J. and Rajeevan,P.K., 2000. Rapid multiplication of phalaenopsis through induction of PLBS from *in vitro* shoots. *Proceedings of the Twenty fifth Kerala Science congress,* Jan. 2000, Kumily. p 501-505

Bhaskar, J. Rajeevan, P.K. and Valsalakumari, P.K. 2001. Rapid multiplication of *Phalaenopsis* through *in vitro* shoot and leaf culture. 6th National seminar on Orchid Diversity in India. 11-13th October, 2001, Palampur. *Abstracts*. p 87

Bhaskar,J., Rajeevan, P.K. and Valasalakumari, P.K. 1999. Effect of sucrose and thiamine HCl on *in vitro* growth of shoot in *Phalaenopsis*. *National Symposia on Emerging scenario in Ornamental Horticulture in 2000 AD and Beyond.* 21-22nd July, 1999. Delhi.

Bhaskar, J., Valsalakumari, P.K., and Rajeevan, P.K. 1996. *In vitro* culture of floral apices of banana. p 531- 533. *Proceedings of the 8th Kerala Science Congress*, Kochi

Chandralekha, K. and Rajendran, P.C. 2000. Controlling browning of *in vitro* cultures of immature inflorescence rachillae of coconut. *Nat. Conf. Recent Trends in Biotechnology*, SD College, Alappuzha, March 25-27

Chandralekha, K. and Rajendran, P.C. 2000. *In vitro* response and causes of recalcitrancy in coconut (*Cocos nucifera* L.). *Natl. Conf. Recent Trends in Biotechnology*, SD College, Alappuzha, March 25-27.

Chandralekha, K. and Rajendran, P.C. 1998. *In vitro* response and causes of recalcitrancy in coconut. *Proc. Nat. Sem. Plant Biotechnology for Sustainable Hill Agriculture, DARL, UP.* p.52

Faizal,, M..H., Susamma Mathai and Peethambaran, C.K. 1996. Efficacy of different spore formulations of *Fusarium pallidoroseum* (Cooke) Sacc in controlling the cowpea aphid *Aphis craccivora* Koch. *Proc.Acad Environ. Biol.* 5: 237-239

Girija, D., Malathy, V.G. and Magu, S.P. 1999. Molecular aspects of lindane degradation by *Pseudomonas* sp. *Nat. Symp. Role of Biochemistry and Biotechnology in the 21st Century.* Bangalore, 6th –8th January 1999.

Girija, D., Nair, P.V. and Mathew, R. 1995. Effect of *Azospirillum* on germination and seedling vigour in rice. *J. Tropic. Agric.* 33:

Girija, D., Malathy, V.G. and Magu, S.P. 2000. Molecular characterisation of a *Pseudomonas* sp. that degrades g - HCH. *Proc. of Twelfth Kerala Science Congress* p.155-159

Girija, D., Nazeem, P. A., Surendragopal, K. and Asha, C. T. 2000. Occurrence of an endophytic and epiphytic nitrogen fixing bacteria in black pepper. (Communicated to *Curr. Sci.*)

Hussain, C.T.S., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1995. Effect of triadimefon and sucrose on *in vitro* corm production in gladiolus cv. Friendship *J. Ornamental Hort.* 3: 59-64

Hussain, C.T.S., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1995. Plant regeneration from root derived callus in gladiolus *J. Ornamental Hort.* 2: 46-50

Hussain, C.T.S., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1997. Regeneration of plantlets through direct organogenesis in gladiolus. Symposium on Tropical crop Research and Development- India International. *Abstracts* ISTCRAD, Trichur Sept-9-10, 1997, *Abstracts.*

Hussain, C.T.S., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1997. Somatic embryongenesis in gladiolus. *National conference on IBMHRD*, Nov 20-22, 1997, Jadavpur University, Calcutta *Abstract* No.39

Hussain, C.T.S., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K., 1998. *In vitro* production on efficient method for faster multiplication of elite clones of gladiolus. *Proc. Tenths Kerala Science Congress*, STEC, Trivandrum, p 243-245

James, D.,Girija, D., Sally K. Mathew and Nazeem, P.A. 2001. Molecular characterization of *Ralstonia solanacearum* causing bacterial wilt in solanaceous vegetables., Paper presented in *Indian Phytopathology Symposium*, Kasargode from 6-8 December 2001.

Jana, M.M., Nadgauda, R.S., Rajmohan, K. and Mascarenhas, A.F. 1994. Rapid somatic embryogenesis from the nucelli of mono-embryonic mango varieties. *In Vitro Plant* 30: 55-57

Jayalekshmi, V. G. and Sree Rangaswamy, S. R. 1999. Genetic finger printing on coconut cultivars. *Proc. Eleventh Kerala Science Congress,* 27 February- 1st March, 1999, Kasargod.

Jiji Joseph, Mallika, V. K and Nair, R.V . 1995. Plantlet regeneration from cocoa (*Theobroma cacao*) somatic embryos through micrografting. *All India Symposium on Recent Advances in Biotechnological Applications of Plant Tissue & Cell Culture*, Bangalore.

Jiji, T., Susamma Mathai, K. B. Soni, S. Simi and K. Rajmohan. 2001. Molecular characterisation of *Fusarium pallidoroseum* (Cooke) Sacc., a biocontrol agent against cowpea aphid. *Insect Environment* 7: 132-133

Karmarkar, S.H, and Keshavachandran, R. 2001. Genetic transformation of *Holostemma adakodien*,K.Schum - a vulnerable medicinal plant. *Journal of Experimental Biology*. 39:1263-1267

Karmarkar, S.H, and Keshavachandran, R. 2002. NAA affects the frequency of hairy root induction in *Holostemma ada-kodien*. *Proc. of the 14th Kerala Science Congress*, 29-31 January 2002. Kochi: 739-740

Karmarkar, S.H, Keshavachandran, R, and Augustin, A. 2001. Biochemical evaluation of root tubers and in vitro induced callus of adapathiyan (*Holostemma ada-kodien* K. Schum) *Journal of Tropical Agriculture* 39: 108-110

Karmarkar, S.H, Keshavachandran, R, Nazeem, P.A, and Girija, D. 2001. Hairy root induction in adapathiyan (*Holostemma ada-kodien* K. Schum). *Journal of Tropical Agriculture* 39: 102-107

Kesavachandran, R. 1997. Anatomical studies of regenerative callus in *Vetiveria zizanioides* (L.)

Nash. Abstracts of the *National Symposium on Emerging Trends in Plant Tissue Culture and Molecular Biology*, Hyderabad. p 86

Kesavachandran, R. 1998. Induction of callus in cashew (*Anacardium occidentale* L.). *Proc. of the Tenth Kerala Science Congress*, January, 1998. Kozhikode p 263-264

Kesavachandran, R. 1998. Screening and evaluation of somaclonal variation in *Vetiveria zizanioides* (L.) Nash. *Proc. of the Tenth Kerala Science Congress,* January, 1998. Kozhikode p 264-265

Kesavachandran, R. , Jaya Manuel and Savitha, A. R. 1997. Regeneration of plantlets through organogenesis in *Tylophora indica* (Burm. F.) Merilt. Abstracts of the *National Symposium on Emerging trends in Plant Tissue Culture and Molecular Biology,* Hyderabad. p 37

Kesavachandran, R. 1996. Morphogenic potential of shoot buds in cashew (*Anacardium occidentale* L.). Paper presented at *PLACROSYM* XII, November, 1996, Kottayam

Kesavachandran, R. 1997. Production of Encapsulated Buds of *Vetiveia zizanioides* (L.) Nash. *Proc of the Ninth Kerala Science Congress.* January, 1997. Thiruvananthapuram. p 1761

Kesavachandran, R. and Md. Abdul Khader, 1996. Callus induction and plant regeneration in *Vetiveria zizanioides* (L.) Nash. *Proc of the Eighth Kerala Science Congress.* January, 1996, Kochi. p 549-552

Kesavachandran, R. and Md. Abdul Khader, 1996. Growth and regeneration of vetiver (*Vetiveria zizanioides* (L.) Nash. Callus tissue under varied nutritional status. *J. Spices and Aromatic Plants,* IISR, Calicut p 60-63

Kesavachandran, R. and Rema Menon, 1997. Control and estimation of phenolic exudation in *in vitro* culture of cashew (*Anacardium occidentale* L.). Abstracts of the *National Symposium on Emerging trends in Plant Tissue Culture and Molecular Biology,* Hyderabad. p 37

Kesavachandran, R. and Shelja, P. B. 1999. Genotypic difference in shoot bud culture of cashew (*Anacardium occidentale* L.). *Proc. of Eleventh Kerala Science Congress.* CPCRI, Kasargode. 27 February- 1st March, 1999

Kesavachandran, R. Jelly Louis 1999. *In vitro* callus inductio in *Coscinium fenestratum* an endangered medicinal plant. *Proc. of Eleventh Kerala Science Congress.* CPCRI, Kasargode. 27 February- 1st March, 1999

Kesavachandran, R. Jelly Louis and Daisy Jacob. 1998. Conservation of rare and endangered medicinal plants through *in vitro* culture. Abstr. Natl. Dialogue. in Management of Plant Genetic Resources, New Delhi. December 1998, p 70

Kesavachandran, R., Asha John and Jayasree, P.L. 1996. Rapid multiplication of *Holostemma annulare* by *in vitro* methods. Abstracts of the *National Symposium on Horticultural Biotechnology,* Bangalore. p 20

Kesavachandran, R., Jelly Louis, C. 2000 *In vitro* propagation of *Terminalia chebula* Retz. *Proc. of Twelfth Kerala Science Congress.* January, Kumali p 569-570

Kesavachandran, R., Jelly Louis, C. 2000 Rapid multiplication of *Habenaria latilabris* through *in vitro* culture. *Proc. of Twelfth Kerala Science Congress.* January, Kumali p 570-571

Kesavachandran, R., Jelly Louis, C., Fathima, A. G. and Sophia John. 2000. Encapsulation of shoot buds of *Holostemma ada-kodien*. *Proc. of Twelfth Kerala Science Congress*. January, Kumali p 568-569

Kesavachandran, R., Ravishankar, G. A and Md. Abdul Khader. 1995. Regeneration of plants from callus cultures of *Vetiveria zizanioides* (L.) Nash. *Recent Advances in Biotechnological Applications of Plant Tissue and Cell Culture*, Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. p 362-370

Kesavachandran, R., Shelja, P. B. and Daisy Jacob, 1999. *In vitro* micrografting in cashew (*Anacardium occidentale* L.) PLACROSYM XIII, Coimbatore, p7

Kesavachandran, R., Sophia A. J., Asha, J. and Bindu, V. 1998. Rapid propagation through shoot bud culture of *Holostemma ada-kodien*. Paper presented at the *International Conference on Medicinal Plants*, February 1998, Bangalore

Keshavachandran. R., Karihaloo.J.L., Nazeem. P.A. and Girija. D. 2002 Genetic diversity studies in *Piper* species through RAPD analysis. Paper presented in *International Symposium on Molecular Approached for Improved Crop Productivity and Quality*, CPMB, TNAU, Coimbatore

Lakshmidevi, S. and Rajeevan, P.K. 1991. Effect of benzyl adenine on shoot proliferation in *Dendrobium spp*. Paper presented at the *National seminar on Biology, Improvement propagation and commercialisation of Indian Orchids* held at IIHR, Bangalore

Lakshmidevi, S., Rajeevan, P.K. and Geetha, C.K. 1993. Clonal propagation of *Dendrobium* spp. through axillary bud release. *Proceedings of the Fifth Kerala Science Congress* p 142-144

Lakshmy, V., P.K. Valsalakumari and P.K.Rajeevan. 1993. Embryo culture in banana hybrids - Mysore (AAB) x Pisnag lilin (AA). *S. Indian Hort*.41: 40

Lalitha, N. A.M.Ranjith and A.Augustin. 2002. Studies on polyphenol oxidase activity and banding pattern in relation to resistance of *Musa* spp.to pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae). *Pest management in Horticultural Ecosystem* 8: 97-102

Lavanya, R., Rajeevan, P.K., Geetha, C.K. and Murali, T.P. 2000. Callus mediated plant regeneration in *Cordyline terminals* (L.) *Kunth National Seminar on Hi-tech Horticulture. Abstracts HSi*, Bangalore – June 26-28, 2000

Leena Ravidas., Valsalakumari, P.K and Rajeevan. P.K. 2001. Seed culture in *Anthurium andreanum. Nat. Symp. Indian Floriculture in the New Millenium.* Indian Society of Ornamental Horticulture, IARI, New Delhi. *Abstracts*. p 76

Leena Ravidas., Valsalakumari, P.K., Rajeevan, P.K and Geetha, C.K. 2002. *In vitro* seed culture in Anthurium breeding. *Proceedings of the Fourteenth Kerala Science Congress*. 29-31 January, 2002, Kochi p 735

Lekshmy, M.L, P.K. Valsalakumari and K. Rajmohan 1992. *In vitro* germination of hybrid seeds of banana. *Agric. Res. J. Kerala.* 30:120-121

Lency Thomas, Nair, R. V, Seeja G.and Mallika, V.K. 1996. Induction of genetic variability in *Vanilla planifolia* through *in vitro* seed culture. *Nat. Symp. Hortic. Biotech.* Oct. 28-30, HSI, Bangalore

Lissamma Joseph, Nazeem P.A, Mini, S. Thampi, Shaji Philip and Mini Balachandran. 1996. *In vitro* techniques for mass multiplication of black pepper (*Piper nigrum* L.) and *ex vitro* performance of the plantlets. *J. Plantation Crops* 24: 511-516

Louis, V., Mathew, J., Nazeem, P.A. and Lissamma, J. 1996. Identification of contaminant bacteria in black pepper tissue culture and its sensitivity to antibiotics. *South Indian Hort.* 42: 43-47

Mallika, V. K, Marymol Manattu, T. Grace Thi-Bin and Nair, R. V. 1996. Field performance of tissue culture derived cocoa (*Theobroma cacao* L) plants from seedling explants. *Nat. Symp. Hortic. Biotech.* Oct. 28-30, HSI, Bangalore.

Mallika, V. K, Rekha, K, Marymol Manattu,, Manjula, M. and Nair, R. V . 1996. Shoot initiation *in vitro* from explants of field grown trees of nutmeg (*Myristica fragrans*). *Nat.Sem. Biotechnology of Spices and Aromatic Plants* (BIOSAP), IISR, Calicut.

Mallika, V.K , Jiji Joseph, Dolly Chacko, Vijayakumar, N.K. and Nair, R. V. 1995. Micrografting - a simple technique for field transfer of tissue culture derived plants in cocoa. *Recent Advances in Biotechnological Applications of Plant Tissue & Cell culture.* G.A. Ravishankar and L.V. Venkataraman (Eds.). 1997. Oxford & IBH, New Delhi

Manju, C.M., L. Rajamony, N. Mohanakumaran and K. Rajmohan. 1996. Embryos of interspecific crosses of *Abelmoschus* rescued *in vitro. Abst.Poster Sess. 2nd Int. Crop Sci. Cong.*, New Delhi p 297

Mary, S, Sangeetha, P. T., Nair R. V and Mallika, V. K. 1998. Effect of mutagens on *in vitro* culture of vanilla (*Vanilla planifolia* Andr.). PLAROSYM XIII, Coimbatore

Mary, S., Lency Thomas, Nair, R. V. and Mallika, V. K. *In vitro* seed culture of vanilla (*Vanilla planifolia*). *J. Plantation Crops* 27: 14-21

Mathew, S. and Rajendran, P.C. 1996. *In vitro* establishment and multiple shoot induction of juvenile and mature clove. *Proc. Eighth Kerala Sci. Congr.* January 1996, Kochi p 232

Mathew, S. and Rajendran, P.C. 2004. *In vitro* callus induction from leaf segments of clove (*Syzgium aromaticum* Merr L. Perry). *Proc.Nat. Sem. Agri-business Cconcept for Sustainable Livelihood,* 6th November 2004, Thrissur (accepted)

Mathew, S. and Rajendran, P.C. 2004. *In vitro* manipulation of problems associated with explant establishment in clove (*Syzgium aromaticum* Merr L. Perry). *Proc.Nat. Sem. Agri-business Cconcept for Sustainable Livelihood,* 6th November 2004, Thrissur (accepted)

Mathew, S.K., Girija, D., Devi, N.S., Sadhankumar, P.G. and Rajan, S. 2000. Variability in isolates of *Ralstnia solanacearum* affecting solanaceous vegetables in Kerala. *Vegetable Sci.* 27:189-191

Mathew, S.K., Girija, D., Binimol, K.S. and James, D. 2002. Characterization of isolates of *Ralstonia solanacearum* causing bacterial wilt of ginger in Kerala. *IV Int. Bacterial Wilt Symp.*, South Africa. 4-6 February p 97

Mini, B., Valsalakumari, P.K. and Geetha. C.K. 1996. Response of various explants of *Musa* AAB Nendran on callus induction and regeneration. p.544. *Proc. Eighth Kerala Sci. Congr.*, Kochi

Mini, B., Valsalakumari, P.K. and Geetha. C.K.. 1995. Effect of repeated subculturing on somaclonal variation in *Musa* sp. var. Nendran. *Journal of Tropical Agriculture.* 33: 125 –127

Mini, B. and Valsalakumari, P.K. 1997. Improvement of propagation efficiency in Nendran banana using eye buds and sucker explants. p 175. *Proc. 9th Kerala Sci. Congr.*, Thiruvananthapuram

Mini, C., M. Abdul Wahab, A. Augustin, S. Rajan. 1998. Standardization of solvent for chilli oleoresin extraction. *South Indian Hort.* 46: 309-311

Mini Raj, A.Augustin and E.V.Nybe. 2001.Biochemical characterization of medicinal plants in the wild and domestic environment. *Proc.Nat. Sem* on Fronters of Research and Development in Medicinal Plants. *Journal of Medicinal and Aromatic Plant Sciences* 22/4A & 23/1A: 623-627

Murali, T.P., Rangan, V.V and Rajeevan, P.K. 1999. *In vitro* propagation of *Cordyline terminalis.Proc.Nat. Sem. Emerging Scenario in Ornamental Horticulture in 2000 AD and Beyond.* 21-22nd July, 1999. Delhi *Abstract* p 14-15

Murali, T.P., Sheeja, K and Rajeevan, P.K. 1999. Micropropagation studies in *Aglaonema. Proc.Nat. Symp. Emerging Scenario in Ornamental Horticulture in 2000 AD and Beyond.* 21-22nd July, 1999. Delhi *Abstract* P.14

Murali, T.P., Sheeja, K., and Rangan, V.V. 1999. *In vitro* protocol development for propagation of *Syngonium podophyllum. Proc.Nat. Symp. Emerging Scenario in Ornamental Horticulture in 2000 AD and Beyond.* 21-22nd July, 1999. Delhi *Abstract* P.16

Murali, T.P., Mathew, S.A. and Rajeevan, P.K. 1997. *In vitro* propagation of *Schefflera arboricola,* an under exploited foliage plant. *Nat. Conf. IBMHRD,* Nov 20-22. Jadavpur University, Calcutta. *Abstracts* No.44

Murugan, C., Sujatha, V.S. and Nazeem, P.A. 2004. Molecular characterization of *Piper* species using RAPD techniques. *Nat. Symp. Rrecent Trends in Applied Biology, Avanashalingam Institute for Home Science & Higher Education for Women.* p 115

Nazeem, P. A., Mathew, S. K., Menon, J. S., Augustine, A., Scaria, S. P. and Joseph, L. 1995. *In vitro* screening of black pepper against the foot rot disease caused by *Phytophthora capsici. Int. Symp. Medicinal and Aromatic Plants, University of Massachusetts Amherst, USA: 7*

Nazeem, P. A., Raji, P., Scaria, S., Joseph, L and Nybe, E. V. 1996. Induction of *Phytophthora* foot rot in black pepper through *in vitro* culture system. *J. Spices and Aromatic Plants*

Nazeem, P. A., Lissamma Joseph, Shaji Philip and E. V. Nybe. 1998. Inductions of variability in ginger (*Zingiber officinale* Rosc.) through indirect organogenesis and *in vitro* mutagenesis. *Proc. Nat. Sem. Plant Biotechnology for Sustainable Horticulture* p 39

Nazeem, P. A, Joseph, L. Rani, T. G., Valsala, P. A.and Philip, S. Tissue culture system for *in vitro* pollination and regeneration of plantlets from *in vitro* raised seeds of ginger (*Zingiber officinale* Rose) 1995. *Int. Symp. Medicinal and Aaromatic Plants, University of Massachusetts Amherst, USA*

Nazeem, P. A. and Menon, R. 1994. Blossom biological and hybridization studies in turmeric (*Curcuma* spp) *S. Indian Hort.* 43: 161-167

Nazeem, P. A., Achuthan, C. R., Babu, T. D. and Jasmine, P.S. 1999. Variability in the protein profile and ß-1,3 glucanase activity in black pepper (*Piper nigrum*) infected with *Phytophthora capsici. Nat. Symp. Role of Biotechnology and Chemistry in the 21st Century*, Bangalore, India p 61

Nazeem, P. A., Geetha, C.K., Joseph, L and Sudhadevi, P.K. 1990. *In vitro* callus induction in black pepper (*Piper nigrum*), *Indian Cocoa Arecanut and Spices Journal* XIV: 34-36

Nazeem, P. A., Joseph, L and Nair, G.S. 1990. *In vitro* plantlet regeneration in black pepper, *Proc. Nat. Symp.Current Trends in Biotechnology, 29*

Nazeem, P. A., Joseph, .L., Geetha, C.K and Nair, G.S. 1990. *In vitro* techniques in cloning of black pepper(*Piper nigrum*), *Proc. Placrosym IX: 64*

Nazeem, P.A., Sudha devi, P.K., Joseph, L. and Geetha, C.K. 1991. *In vitro* production from *Gymnema Proc. 11th Kerala Sci. Congr.* p 182-183

Nisha, K.K., K. Seetha, K. Rajmohan and M. G. Purushothama. 2003. *Agrobacterium tumefaciens* mediated transformation of Brahmi [*Bacopa monniera* (L.) Wettst.], a popular medicinal herb of India. *Curr. Sci.* 85 (1): 85-89

Peter, K. V., Nazeem, P.A., Ravindran, P.N and Nirmal Babu, K. 2001. Application of Biotechnology for the improvement of spices. *Nat. Sem. Biotechnology – challenges and prospects.* St. Aloysius College, Mangalore, Januray 4-6

Peter, K.V., Keshavachandran, R., Nazeem, P.A. and Mallika, V.K. 2000. Biotechnology of plantation crops and spices. *The Botanica* 50: 23-32

Prabha, J. and Kesavachandran, R. 1995. *In vitro* multiplication and field establishment of *Ananas comosus* (L.) Merr. *Recent Advances in Biotechnological Applications of Plant Tissue and Cell Culture.* Oxford & IBH Publishing Co. Pvt. Lts. New Delhi. p 126-131

Prasannakumari Amma, S, Sreekala, C, Mallika, V. K, Nair, R. V. and Nybe, E. V. 1996. *In vitro* induction of multiple plantlets from single zygotic embryo of coconut *(Cocos nucifera* L). *Nat. Symp. Hort. Biotech.,* Oct. 28-30, Bangalore, (Abstract)

Priyakumari, I, V.L. Sheela and K. Rajmohan. 2002. *Micropropagation of gladiolus* cv. Tropic Seas. *Floriculture Research Trend in India* (*eds.* R. L Misra and Sanyat Misra). Indian Society of Ornamental Horticulture, New Delhi. p 91-94

Radhika, N.S. and Umamaheswaran, K. 2000. Etiology of a severe mosaic of cowpea and its management through genetic and biochemical approaches. *Proc. 12th Kerala Science Congress,* Kumily p 346-366

Rajeevan, P.K., Sheeja, K., Rangan, V.V. and Murali, T.P. 2002. Direct organogenesis in *Syngonium podophyllum . Nat. Symp. Indian Floriculture in the New Millenium.* Indian Society of Ornamental Horticulture, IARI, New Delhi. *Abstracts* p 85

Rajeevan, P.K., Valsalakumari, P.K., Geetha, C.K. and Sudeep, R. 1997. Shoot production in *Dendrobium* as influenced by palm milk and floral extracts of *Spathodia, Nat. Conf.* IBMHRD, Nov 20-27, Jadavpur University, Calcutta. *Abstracts*

Rajendran, P.C. and Thara, K.M. 2004. Multiple shoot induction of triploid seeds and anther culture in watermelon (*Citrullus lanatus* Thumb.). *Proc.Nat. Sem. Agri-business Concept for Sustainable Livelihood,* 6th November 2004, Thrissur

Rajendran, P.C. and Suma, B. 2004. Eco-friendly and biotechnology linked Agri-business entrepruenership for rural livelihood. *Proc.Nat. Sem. Agri-business Cconcept for Sustainable Livelihood,* 6th November 2004, Thrissur

Rajendran, P.C., Indu C Nair, Sheeja, K. and Nybe, E.V. 1998. Induction and growth of callus derived from immature endosperm of *Carcinia cambogia* Desr. *Proc. Nat. Sem.Plant Biotechnology for Sustainable Hill Agriculture.* Defence Agriculture Research Lab, UP, May 5-8, p16

Rajendran, P.C., Manju, T.G. and Sreekanth, P.M. 2004. Somatic embryogenesis through *in vitro* endosperm culture in kodampuli (*Garcinia gummi-gutta* var. gummigutta). *Proc.Nat. Sem. Agri-business Cconcept for Sustainable Livelihood,* 6th November 2004, Thrissur

Rajendran, P.C., Manju, T.G., Sreekanth, P.M. and Sunil Nair, A. 2004. Caulogenesis and rhizogenesis from nodal segments and shoot tips of *Garcinia gummi-gutta* var. gummigutta. *Proc. 12th Kerala Science Congress,* Peechi

Rajendran PC, Sreekanth PM and Manju TG (2004). Breaking seed dormancy and sex detection through biotechnological pathway in *Garcinia gummi-gutta* var. gummigutta) *Proceedings of the National Seminar on Agri-business concept for sustainable livelihood,* 6th November2004, Thrissur (accepted)(abstract)

Rajendran, P.C., Sreekanth, P.M and Manju, T.G. 2004. Isolation of protoplasts from juvenile leaf of the *Garcinia gummi-gutta* var. gummi-gutta. *Proc.Nat. Sem. Agri-business Concept for Sustainable Livelihood,* 6th November 2004, Thrissur

Rajendran, P.C., I.C. Nair, P.K. Sheeja and E.V. Nybe. 1998. Induction and growth of callus derived from immature endosperm of *Garcinia cambogia* Desr. *Proc. Nat. Sem. Plant Biotechnology for Sustainable Hill Agriculture, DARL, UP.* p.16

Rajmohan, K. and N. Mohanakumaran. 1990. Callus culture and organogenesis in black pepper (*Piper nigrum* L.). *Abst. VII Intl. Congr. Plant Cell and Tissue Culture,* Amsterdam. p 127

Rajmohan, K. and N. Mohanakumaran. 1988. Influence of explant source on the *in vitro* propagation of jack (*Artocarpus heterophyllus* Lam.). *Agrl. Res. J. Kerala* 26: 169-174

Rajmohan, K. 1999. Advances in biotechnology with special reference to Kerala. *Proc.Nat. Sem.*

Participatory Approaches for Horticultural Development, Kerala Horticultural Development Programme, Kochi. p.45-46

Rajmohan, K. and N. Mohanakumaran. 1996. Regulation of phase change in perennials in relation to *in vitro* propagation and improvement. *Abst.Poster Sess. 2nd Int. Crop Sci. Cong.,* New Delhi

Rajmohan, K. and N. Mohanakumaran. 1996. Overcoming the maturity barrier in the *in vitro* propagation of perennial plant species. *Proc. 8th Kerala Sci. Cong.,* Kochi p.547-548

Rajmohan, K. and N. Mohanakumaran. 1983. *In vitro* rooting of jack shoot cultures. *Agrl. Res. J. Kerala* 21: 53-55

Rajmohan, K. and N. Mohanakumaran. 1988. Effect of plant growth substances on the *in vitro* propagation of jack (*Artocarpus heterophyllus* Lam.). *Agrl. Res. J. Kerala* 26: 29-34

Rajmohan, K. and N. Mohanakumaran. 1988. Studies on the *in vitro* propagation of mussaenda *via* somatic organogenesis/embryogenesis. *Agrl. Res. J. Kerala* 26: 9-16

Rajmohan, K. and N. Mohanakumaran. 1989. *In vitro* propagation of tree crops of horticultural importance: problems and prospects. *Proc. Natl. Symp. Recent Advances in Plant Cell Research.* University of Kerala, Trivandrum

Rajmohan, K., N. Mohanakumaran and N. Mohanbabu. 1989. Application of tissue culture for the improvement of tree crops. *In vitro Approaches to Breeding* (ed. Varghese, J.P.). C M.S. College, Kottayam. p 119-126

Rajmohan, K., S. Sadheeshnakumari and S. Suneetha. 1999. *In vitro* clonal propagation of *Aristolochia indica* Linn. *Proc. 11th Kerala Sci. Cong.,* Kasaragod p.225-226

Rajmohan, K., S. Sadheeshnakumari, S. Suneetha and G. Sreekandan Nair. 1997. An improved protocol for the *in vitro* propagation of *Vanilla plannifolia. Proc. 9th Kerala Sci. Cong.,*Trivandrum p187

Ramesh, B., K. Rajmohan and N. Mohanakumaran. 1992. *Ex vitro* rooting of jack (*Artocarpus heterophyllus* Lam.) microcuttings. *Abst. Intl. Symp. on Transplant Production Systems*, Yokohama. p1

Ramesh, B., K. Rajmohan and N. Mohanakumaran. 1993. *Ex vitro* establishment of jack plantlets: basic and applied aspects. *Proc. 5th Kerala Sci. Cong.*, Kottayam, p 106-108

Ramesh, P. and Rajmohan, K. *In vitro* propagation of polyembryonic mango (*Mangifera indica* L.) varieties of Kerala. *Proc. 11th Kerala Sci. Cong.*, Kasaragod

Reena Kartha, V, Mallika, V. K, Minu Joseph, Lency Thomas and Nair, R. V. 1996. *In vitro* mutagenesis in vanilla (*Vanilla fragrans*) using gamma irradiation. *Nat.Sem. Biotechnology of Spices and Aromatic Plants* (BIOSAP), IISR, Calicut

Renjith, D. and Valsala, P.A. 2001. Study on variability of quality components in turmeric (*Curcuma longa* L.). *Proc. 13th Kerala Sci. Cong.*, Thrissur, p 437

Renjith, D., Valsala, P. A. and Nybe, E.V. 2001. Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination. *J. Spice. Aroma. Crop* 10: 135-139

Sankar, M. A. and Nair, G.S. 1997. Callus induction in *Sida spp. Proc. 9th Kerala Sci. Cong.*, Thiruvananthapuram p 196-197

Sankar, M.A and Nair, G.S. 1997. Effect of cytokinin on callusing in *Sida* spp. *Souvenir and abstract of National Symposium on emerging trends in Plant Tissue Culture and Molecular Biology* held at Osmania University, Hyderabad, p.36-37

Sankar, M.A and Nair, G.S.1999. Somatic organogenesis in *Sida* spp. *Proc. 87th Session of the Indian Sci. Cong.*, Pune, p.115-116

Sankar, M.A, Nair, G.S. and Augustine, A. 2000. Ephedrine synthesis in *in vitro* culture of *Sida* spp. through precursor feeding. *J. Med. Arom. Pl. Sci.* 22/4A: 248-251

Sareena, S. Kurien and .Augustin, A. 2000. Anatomical and Biochemical Studies during rooting of cuttings in baduvapuli (*Citrus pennivessiculata* Tan.), Proc. ISC Cong. 2000, 3-7 Dec, Orland, Florida, USA.128

Sebastine, A. and V. S. Sujatha. 1996. Isoenzyme variation in different plant parts and at different stages of leaf development in *Piper nigrum* L. *J. Trop. Agri.* 34: 136-37

Sebastine, A., V. S. Sujatha, E. V. Nybe, G. S. Nair and A. Augustine. 1996. Isoenzyme variation and species relationship in the genus *Piper. J. Trop. Agri.* 34: 85-92

Seema, B, J, and Kesavachandran, R. 1998. Rapid propagation through *in vitro* culture of *Trichopus zeylanicus* Gaerth. *Abst. Nat. Sem.Plant Biotechnology for Sustainable Hill Agriculture*, May 1998, Pithoragarh p 14

Seema, B. J., Kesavachandran, R. and Mini Joseph. 1998. *In vitro* propagation of *Trichopus zeylanicus* Gaertin., an endangered medicinal plant. Paper presented at the *Int. Conf. Medicinal Plants*, February 1998, Bangalore

Sethunathan, N., Sahu, S.K. and Girija, D. 1998. Bioremediation of organochlorines in a rice cropping system. Paper presented in the ACIAR sponsored *Asia-Pacific Workshop on Seeking Agricultural Produce Free of Pesticide Residues,* Indonesia, 17-19 Feb 1998

Shammy Mathew and Rajendran, P. C. 1996. *In vitro* establishment and multiple shoot induction of juvenile and mature clove. 1996. *Proc. 8th Kerala Sci. Congress,* January p 232

Sheeja, G. Jisha, K.G., Seetha, K. Joseph. L and Nair, R.V. 1998. Tissue culture of Cinnamon (*Cinnamomum verum* PRESL.). PLAROSYM XIII, Coimbatore

Sheela, V.L. and Rajmohan, K. 2000. Uptake of nutrients by tissue cultured plants of banana *Musa* (AAB) 'Nendran'. Paper presented in *Nat. Symp. Recent Trends in Plant Science Research,* April 17-19, 2000, Univ. Kerala, Thiruvananthapuram

Sheela, V.L. and Nair, S.R. Nutrient requirement of tissue culture banana in comparison with sucker produced banana *Musa* (AAB) 'Nendran'. Paper presented in *Symp. Technological Advancement in banana / plantain production and processing,* 20-24 August 1996, Thrissur

Sheela, B., George,T.E, Peter, K.V. and Augustin, A. 2001. Oleoresin and ascorbic acid content in Bird Pepper (*Capsicum frutescens* L.) as influenced by maturity. *Proc. 13th Kerala Sci. Congress,* January 2001,Trissur p 449-450

Shylaja, M. R. and Nair, G. S. 1996. Somaclonal variation in black pepper cultivars for *Phytophthora* foot rot disease reaction. Paper presented in the *Nat. Symp. Horticultural Biotechnology.* 28-30 Oct. 1996, I.I.H.R. Bangalore. p 39-40

Shylaja, M. R and Nair, G. S. 1998. Effect of callus screening with toxic metabolite(s) of *Phytophthora capsici* on morphogenesis in black pepper (*Piper nigrum* L.) *Proc. Nat. Sem. Plant Biotechnology for Sustainable Hill Agriculture,* DARL, Pithoragarh

Shylaja, M. R., Nair, G. S. 1997. Plantlet regeneration from calli screened against *Phytophthora* foot rot in black pepper. *Proc. 9th Kerala Sci. Congress,* Trivandrum, p 197-198

Shylaja, M. R., Nair, G. S. and Babu, L. C. 1997. Root growth and root anatomy of regenerants derived from calli screened against *Phytophthora* foot rot disease in black pepper *Piper nigrum* L. Paper presented in the *Nat. Symp. Emerging Trends in Plant Tissue Culture and Molecular Biology.,* 29-31 January, 1 997 , Osmania University, Hyderabad. p 82

Shylaja, M. R., Nair, G. S. and Mathew, J. 1996. Screening of black pepper (*Piper nigrum* L.) calli clones for *Phytophthora* foot rot resistance / tolerance. *J. trop. Agric.* 34: 115-120

Shylaja, M. R., Nair, G. S., Nazeem, P. A. Mallika, V. K. and Mathew, J. 1996. *In vitro* screening of black pepper (*Piper nigrum* L.) for tolerance to *Phytophthora capsici. J. Pln. Crops* 24 (Supplement) : 171-178

Shylaja, M. R., Nair, G. S., Augustine, A and Mathew, J. 1997. *In vitro* production of toxic metabolite(s) by *Phytophthora capsici* and partial purification of the metabolite(s). *J. trop. Agric.* 35: 10-15

Shylaja, M.R,Sanju.C.R.,Resmi Paul and Nybe.E.V. 2003. *In vitro* adventitious bud regeneration in ginger (*Zingiber officinale* Rosc.); response of cultivars. Paper presented in *National Seminar on New Perspectives in Spices, Medicinal and Aromatic Plants*, ISS, Goa. p 51-55

Sindhu, M and Keshavachandran, R. 2002. Enhancement of berberine in the liquid medium of *Coscinium fenestratum* cell suspension cultures. *Proc. of the 14th Kerala Science Congress*, 29-31 January 2002. Kochi: 373-376

Sindhu, M. and Kesavachandran, R. 2000. Callus culture of *Coscinium fenestratum*, a berberine yielding species. *Proc. 12th Kerala Science Congress*, January, Kumali p 437-440

Sindhu, S., Kesavachandran R., Nybe, E.V. and Augustin, A. 2000. Isolation and estimation of Berberine from *in vitro* and *ex vitro Coscinium* plant samples. Nat. Symp. Medical, Plant & Industrial Biotechnology, Cochin University of Science & Technology, 1-2 December 2000, Kochi, 25

Sivaprasad, P., B. Ramesh, N. Mohanakumaran, K. Rajmohan and P.J. Joseph. 1995. Vesicular arbuscular mycorrhizae for the *ex vitro* establishment of tissue culture plantlets. *Proc. Third Nat. Conf. on Mycorrhiza*. p 281-283

Sivaprasad, P., Thomas Bennan, S. Suneetha and K. Rajmohan. 1998. Synergistic effect of arbuscular mycorrhizal fungi and *Azospirillum* on tissue culture plantlets. *Phytopathol.* 88:82

Sobhana, A. and Rajeevan, P.K. 2002. Refinement of embryo culture medium in *Dendrobium*. *Nat. Symp. Indian Floriculture in the New Millenium*. Indian Society of Ornamental Horticulture, IARI, New Delhi. *Abstracts*. p 68

Sobhana, A. and Rajeevan, P.K. 2004. Effect of irradiation on the *Dendrobium* hybrid Emma White x Banyat Pink. *Nat. Symp. Recent Trends and Future Strategies in Ornamental Horticulture*. 1-4 December, 2004. *Abstracts*. p 21

Sobhana, A. and Rajeevan, P.K. 2004. *In vitro* callusing *in Dendrobium* protocorons. *First Indian Horticulture Congress*, 2004, New Delhi. *Abstracts*.p 160-6

Sobhana, A. and Rajeevan, P.K. 1997. Clonal multiplication of *Epidendrum* through meristem culture. *Nat. Conf. IBMHARD*, Nov. 20-22, Jadavpur University, Calcutta, Abstracts 115

Sobhana, A. and Rajeevan, P.K. 2003 . Effect of sucrose and charcoal on seedling growth of New Pink x Emma White, a *Dendrobium* hybrid. *Nat. Symp. Recent Advances in Indian Floriculture*. ISOH, IARI, New Delhi. *Abstracts* p 67

Sobhana, A. and Rajeevan, P.K. 2003 . *In vitro* seed germination studies in *Dendrobium* hybrids. *One hundred Research Papers in Floriculture: Nat. Symp. Recent Advances in Indian Floriculture*. ISOH, IARI, New Delhi. *Abstracts* p. 231-233

Sophia John, A. and Kesavachandran, R.. 1996. Plant Regeneration in *Holostemma annulare* through shoot bud culture. *Proc 8th Kerala Science Congress.* January, 1996, Kochi

Sreelatha, U., S. Ramachandran Nair and K. Rajmohan. 1998. Factors affecting somatic organogenesis from leaf explants of *Anthurium* species. *J. Ornamental Horticulture*, New Series 1: 48-54

Sreelatha, U., S. Ramachandran Nair and K. Rajmohan. 1999. *In vitro* multiple shoot formation in *Anthurium andreanum*. *South Indian Hort.* 42: 348-52

Sudeep, R., Rajeevan, P.K. and Geetha, C.K. 1994. Influence of amino acid supplements on shoot proliferation in *Dendrobium*. *Nat. Sem. A Decade of Orchid Research and Development.* October 24-25, New Delhi. Abstracts p 79

Sudeep, R., Rajeevan, P.K., Valsalakumari, P.K. and Geetha, C.K. 1996. Influence of cytokinin related substance on shoot proliferation in *Dendrobium*. *Nat. Sem. Propagation and Nursery Management*, 4-5 October, Anand

Sudeep, R., Rajeevan, P.K., Valsalakumari, P.K. and Geetha, C.K. 1996. Influence of organic supplements on shoot proliferation in *Dendrobium*. *Nat. Sem. on Propagation and Nursery Management.*, 4-5 October, 1996. G.A.U., Anand. Abstracts. p 38

Sudhadevi, P.K., Geetha, C.K. and Radhakrishna, T.C. 1993. *In vitro* regeneration of plantlets from hybrid seed embryo of pineapples (*Ananas comosus* (Linn) Merr). Paper presented in the *Nat. Symp. Horticulture Research: Changing Scenario* p 462

Sudhadevi, P.K., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1995. Performance of *in vitro* developed pineapple plantlets. *South Indian Hort.* 43: 122-124

Sudhadevi, P.K., Geetha, C.K., Rajeevan, P.K., Valsalakumari, P.K. and Radhakrishna, P.C. 1994. Rapid conal propagation of pineapple. (*Ananas comosus* L) through *in vitro* techniques. *South Indian* Hort. 2 : 5-10

Sulekha, G. R., K. Rajmohan, S. Suneetha and G. S. Nair. 1996. Rapid *in vitro* clonal propagation of *Musa* (AAA) 'Red Banana'. *Abst. Symp. Technological Advancement in Banana / Plantain Production and Processing*, Thrissur. p 13

Sulekha, G. R. and K. Rajmohan. 1999. *In vitro* somatic embryogenesis in mango (*Mangifera indica* L.). *Nat. Sem. Participatory Approaches for Horticultural Development*, Thiruvananthapuram. p 53

Susha, S., Thara, K. M. and Naseema, A. 1998. Biocontrol of water hyacinth using fungal pathogens. *Proc. 10th Kerala Science Congress*, 2-4. January, Kozhikode p 362-365

Thara, K. M, Sindhu, K., Lissymol,V., Nazeem P. A. and Nair, R.V. 1998. *In vitro* techniques for the micropropagation of elite and dwarf plants of clove (*Syzygium aromaticum* L.). PLAROSYM XIII, Coimbatore

Thara, S. S. and Naseema, A. 1997. Mass production of *Fusarium* spp., potential biocontrol agents of water hyacinth. *Nat. Sem. Sustainable Environment*, Nov. 4-6, Alwar Kuruchii

Thara, S. S. and Naseema, A. 1998. Biocontrol of water hyacinth using fungal pathogens. *Proc. 10th Kerala Science Congress*, Kozhikode. p 362-365

Ullas Mony and K. Rajmohan. 1999. Apomixis in relation to agricultural production. *Abst. Nat. Sem. Role of Biochemistry and Biotechnology in Twenty First Century*, Bangalore. p 120

Umamaheswaran, K. and Balakrishnan, S. 1985. Serological studies of pumpkin mosaic virus in Kerala. *Indian J. Mycol. Pl. Pathol.* 15: IX

Uma Maheswari, R., T. Sabeena and K. Rajmohan. 2003. *Ex vitro* nutrition of *Dendrobium* Sonia 17 plantlets. *Nat. Symp. Recent Advances in Indian Floriculture*, November 12-14, Thrissur. Abstract I-108

Valsala, P. A and Nair, G. S. 1996. Hybridization in *Zingiber officinale* (Rosc.) through *in vitro* pollination. *Proc. 8th Kerala Science Congress*, Thiruvananthapuram, p 119-112

Valsala, P. A, and Nair, G. S.. 1996. Structure of ginger seed produced under *in vitro*. Proc. Nat. Symp. Horticultural Biotechnology, Bangalore

Valsala, P. A, Nair, G. S. and Nazeem, P. A. 1996. Seed set in ginger (*Zingiber officinale* Rosc.) through *in vitro* pollination. *J. Trop. Agric.* 34: 81-84

Valsala, P. A, Nair, G. S. and Nazeem, P. A. 1998. *In vitro* seed set and seed development in ginger, *Zingiber officinale* Rosc. *Proc. Nat. Sem. Biotechnology of Spices and Aromatic Plants (BIOSAP)* Calicut, Kerala

Valsala, P.A. and Nair, G.S. 1998. Culture establishment and *in vitro* pollination of ginger (*Zingiber officinale* Rosc.) flowers. Souvenir, *Nat. Sem.Plant Biotechnology for Sustainable Hill Agriculture*. Defence Research and Development organization, Pithragarh

Valsala, P.A., Nair, G.S. and Suresh Babu, K.V. 2001. *In vitro* pollination in ginger-proof of fertilization. *Nat. Sem. Hi-tech Horticulture*. New Delhi,

Valsalakumari, P.K., Musthafa, M.S., Geetha, C.K. and Rajeevan, P.K. 1997. Field performance of micropropagated Nendran (*Musa* AAB) product by serial subculturing. *Symp. Tropical Crops Research and Development*, Trichur.

Valsalakumari, P.K., P.K.Rajeevan and Lekshmy, M.L. 1996. Embryo culture in banana. *Proc. 8th Kerala Science Congress*, Kochi p 183-184

Valsalakumari,P.K., M.L.Lekshmy, K. Rajmohan and P.K.Rajeevan. 1990. Embryo culture in banana. *Banana Newsletter* 56: 58

Viji, M.M., M. Thangaraj and M. Jayapragasam. 1996. Studies on the progenies of embryo culture derived rice plants for salinity tolerance. *Oryza* 33: 6-10

Viji, M.M., M. Thangaraj and M. Jayapragasam. 1997. Molecular aspects of low light stress tolerance in rice (*Oryza sativa* L.). *Biologia Plantarum* 39: 251-256

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