UNIT FOR BIOTECHNOLOGY IN AGRICULTURE

I. INTRODUCTION

The need for technological advancement in Agriculture is increasingly felt at present. Biotechnology offers scope for vertical increase in productivity. Rapid advancement in the various frontiers of plant biotechnology can revolutionise Indian Agriculture, bringing in another Green Revolution.

The Kerala Agricultural University is set to exploit this promising field. The present proposal aims at exploiting the remarkable progress achieved elsewhwere in the different fields of biotechnology, relevant to Kerala context.

Plant biotechnology research was initiated at the Kerala Agricultural University in 1981 with the establishment of a Plant Tissue Culture facility at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara. The primary emphasis of the Laboratory, then, was micropropagation of horticultural crops. Successful methods of *in vitro* propagation were standardised for jack, cardamom, mussaenda and bougainvillea. In the case of jack, cardamom and banana, plantlets could be established in soil. Preliminary studies on the culture establishment of pepper, nutmeg and breadfruit were made. Micropropagation of cocoa, pepper and ginger is being attempted. Embryo culture and *in vitro* mutagenesis of banana were also carried out. The facilities/equipment available are the bare minimum. The Laboratory has one culture room, one inoculation room, one media preparation room, laminar air flow chamber, growth chamber and other minor items of equipment. Five scientists and two PG students are working.

Plant Tissue Culture facility was created in the Department of Pomology and Floriculture in 1988 to work on *in vitro* propagation of rare orchids. The facilities include a small culture room-cum-inoculation room and one media preparation room. Two scientists and one PG student are working.

At the College of Agriculture, Vellayani a Plant Tissue Culture Laboratory was established in 1988 with the financial support from FERRO. Studies on the *in vitro* propagation of cashew and nutmeg are in progress at this centre. It has the essential facilities for *in vitro* propagation work. The facilities include two culture rooms, one inoculation room, one media preparation-cum-staff room, one computer room, autoclave, growth chamber, shaker, laminar air flow chamber, deep freeze, electronic balance, camera unit, slide projector and other essential items of minor equipment. Four scientists and five PG students are working.

The projects on biotechnology ongoing in the KAU are listed below:

1. *In vitro* propagation of nutmeg and cashew (FERRO project)
2. *In vitro* propagation of cocoa clones
3. *In vitro* propagation of orchids
4. *In vitro* propagation of banana
5. *In vitro* propagation of anthurium
6. *Ex vitro* establishment of jack plantlets
7. Culturing flat bean embryos of cocoa for recovery of haploids
8. Anther culture in cocoa
9. *In vitro* mutagenesis in banana.
10. *In vivo* and *in vitro* mutagenesis in rose

The present proposals aim at establishing an Advanced Centre for Plant Biotechnology Research at the Kerala Agricultural University, with a main centre at Vellanikkara and a sub centre at Vellayani.

The Centre would work on the following immediate priority areas:

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1. *In vitro* propagation
2. *In vitro* genetic improvement
3. Basic studies involving Molecular Biology & Biochemistry
4. *In vitro* secondary plant metabolite production
5. *In vitro* preservation of germ plasm
6. Genetic modification of microbes
7. Breaking the immune response of insect pests to pathogens
8. Mass multiplication of insect pathogens/beneficial organisms
9. Pheromone production

A core staff will be provided to manage the centres. In addition to research, they will be responsible for offering post-graduate students'programmes and for imparting training, both at technician and higher levels. At the Vellanikkara main centre, one Professor, two Associate Professors, three Assistant Professors and sopporting staff have been proposed. At the Vellayani sub centre, one Associate Professor, two Assistant Professors and supporting staff have been proposed.

The Centre is proposed as a common facility for the scientists of the various Departments involved in biotechnology research. The infrastructure and other facilities have been proposed with scope for future expansion. The major part of the research programmes envisaged under the proposals will be carried out by the scientific personnel already available at the Kerala Agricultural University. Provision has been made for short term training programmes for scientists and technical personnel.

II. PROGRAMMES

A. RESEARCH AREAS

1. *In vitro* propagation of species of economic importance

In vitro clonal propagation is increasingly being used as an alternative means of asexual propagation of economically important plants. It ensures very rapid rate of multiplication and is not season-dependent. Only a limited plant material is required as the initial explant. Micropropagation can speed up the process of establishing new varietis. It is of immense importance when effective conventional means of vegetative propagtion are lacking. *In vitro* raised clonal plants are reported to grow faster and to mature earlier than the seed propagated plants. Selective propagation of dioecious genotypes,maintenance of male sterile genotypes and inbred lines and multiplication of heterozygous genotypes are possible using *in vitro* methods of clonal propagation.

In the *in vitro* propagation *via* enhanced release of axillary buds, the dormant buds of a vegetative apex are stimulated in the presence of cytokinins to grow and elongate. Such shoots are either allowed to root (*in vitro* or *in vivo*) or used for further propagation by excision of the terminal and axillary buds. Shoot apex culture is sufficiently productive in that millions of plants can be generated annually. Genotypic stability is generally maintained among the progenies.

In vitro propagation *via* somatic embryogenesis has greater multiplication efficiency. Further, as root and shoot growth occurs simultaneously in the embryoids, separate shoot growth and rooting steps are not necessary. It is believed that cells from any plant, given the appropriate stimuli and conditions, could be induced to follow the embryogenetic pathway. At present, success is reported in a few species belonging to different families, indicating that it is not limited to a few taxa. Somatic embryogenesis can be used for the rapid multiplication of immature interspecific hybrid embryos. Possibility exists for large scale production of somatic embryos and their encapsulation with suitable gel, to produce artificial seeds. Because of their inherent properties as embryos, somatic embryoids may be useful for longterm storage. They may alternatively be used for the *in vitro* production of flavour compounds. Direct somatic organogenesis, though reported in a very few instances at present, can also be resorted to for *in vitro* clonal propagation.

Attempts for standardising the *in vitro* propagation procedures have not been made for most of the important crops of Kerala. In cases where procedures have been standardised, further improvement in the *in vitro* response is possible by optimising the culture media, culture conditions and pre-culture treatments.

Micropropagation of crop plants was initiated at the Kerala Agricultural University in 1981. Procedures were standardised for the *in vitro* clonal propagation of jack, cardamom, mussaenda and bougainvillea. Efforts are underway in the case of cashew, nutmeg, cocoa, banana, ginger, turmeric, pepper, anthurium and rare orchids.

In the case of jack, plantlets could be produced *via* enhanced release of axillary buds from shoot apices of fresh stem sprouts of five, ten and thirty-year old trees. The MS medium supplemented with GA 1.0 ppm and activated charcoal 1.0% was identified as the suitable culture establishment medium, in which the explants were incubated in darkness for four weeks with repeated subculturing. The cultures were then exposed to light for two weeks, after which the growing shoot apices were transferred to the proliferation medium (MS + BA 5.0 ppm + NAA 0.2 ppm + adenine sulphate 20 ppm + insoluble PVP 500 ppm). Shoots from the proliferation medium were transferred after five weeks to an elongation medium (MS + BA 2.0 ppm + NAA 2.0 ppm + insoluble PVP 500 ppm). The shoots were then cultured on MS medium containing activated charcoal 1.0% for two weeks. For *in vitro* root induction, the shoots were cutured in darkness in half MS + IBA 2.0 ppm + NAA 2.0 ppm (for six days) and 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 (3500 lux) for one week. The plantlets were then transferred to vermiculite medium and subjected to a gradual hardening process before transferring to garden pots kept in the open field conditions. Shoot apices from seedlings registered a multiplication rate of 17.4x and rooting percentage of 100. Explants from five-year old trees had a shoot multiplication rate of 4.5 and produced 70% rooting. Explants from ten-year old trees had a shoot multiplication rate of 2.8 and rooting percentage of 40. Explants from thirty-year old trees recorded a shoot multiplication rate of 2.09 and rooting percentage of 15. Explants from six-month old grafts failed to produce multiple shoots and exhibited 50% rooting. Cytological examination revealed the stability of chromosome number in the plantlets.

In the case of cardamom, multiple shoots were induced from rhizome buds cultured on MS liquid medium supplemented with cytokinins, auxins and coconut water. The study indicated a rate of shoot multiplication of 10x within a culture period of twelve weeks.

In vitro propagation of mussaenda could be achieved *via* callus-mediated somatic organogenesis and embryogenesis as well as enhanced release of axillary buds. Ovary wall segment was identified as the best source of explant for profuse callus production when cultured on MS + NAA 2.0ppm + kinetin 1.0ppm. Shoot regeneration from the callus occurred on MS medium with BA 2.0ppm or BA 0.5ppm + kinetin 0.5ppm. Root regeneration was on MS medium containing NAA 8.0ppm and kinetin 2.0ppm after 60 days of culture. Somatic embryoids were observed to develop when the

callus from the induction medium (MS + 2,4-D 2.0 ppm + kinetin 1.0 ppm) was transferred to MS medium containing BA 0.5 ppm + kinetin 0.5 ppm after 73 days of culture.

Profuse callus production was obtained in the case of bougainvillea on MS medium supplied with NAA 1.0 ppm and BA 0.5 ppm. Explants.(shoot apices and immature axillary buds) from the variety "Mahara" responded better than those of "Cherry Blossom" or "Spring Festival".

The proposal aims at the in vitro clonal propagation of species of economic importance to Kerala. Standardisation of techniques for the in vitro propagation and ex vitro establishment of plantlets and the evaluation of their field performance are envisaged. All the three routes of in vitro propagation (enhanced release of axillary buds, direct/callus mediated somatic embryogenesis and direct/callus mediated somatic organogenesis) will be tried for the purpose.

The following crops will be considered. In each case, the progress so far achieved has been briefly reviewed and the proposed plan of work outlined.

Cocoa

Old and ageing plantings raised from inferior and heterogenous quality seedlings contribute to the major share of cocoa production in the world. Large scale clonal multiplication of superior genotypes alone can substantially increase the productivity. Micropropagation is useful in this context.

Progress of work in other centres

Preliminary reports on the in vitro propagation of cocoa were made by Orchard et al., 1979 and Pence et al.,1979. Passey and Jones,1983 succeeded in shoot proliferation and rooting of non woody nodal bud explants of three-year old Amelonado cocoa seedlings. Adu-Ampomah et al., 1987 presented a protocol for the production of plantlets by shoot apex culture using explants from germinating mature beans in vitro.

Reports of somatic embryogenesis were presented by Esan (1975) and Pence et al. (1979 and 1981) using cotyledons, hypocotyl tissues and from the tip of glandular hair like structures of cocoa. Adu-Ampomah et al.(1987) developed a protocol for the production of plantlets via somatic embryogenesis using immature beans of cocoa. Cultivar differences were observed in the ability to produce somatic embryoids.

Progress of work in KAU

In vitro propagation of cocoa *via* enhanced release of axillary buds was attempted using nodal cuttings from field grown plants as per the methods suggested by Orchard *et al*., 1979. Bud elongation and swelling followed by the production of upto two fully expanded leaves after two months were observed. However, subculturing of the shoots failed due to bacterial contamination. Later, attempts for bud culture failed due to fungal contamination from the explants, regardless of the procedures used to sterilise the stem fragments. Efforts are being made for establishing sterile cultures.

Shoot tip culture of cocoa from sterile plants raised from mature beans *in vitro* as per the protocol of Adu-Ampomah *et al*., 1987 proved successful. Multiple shoots were induced and the individual shoots were rooted *in vitro*. Attempts for planting out are in progress. Efforts are being made to produce somatic embryoids.

Proposed programme

In vitro propagation of cocoa will be attempted via enhanced release of axillary buds and somatic embryogenesis using mature explants for the rapid multiplication of proven hybrids and selected plants. *Ex vitro* establishment of the plantlets and the long term field evaluation of their performance will also be undertaken.

Coconut

The present genetic stock of coconut is of poor and inferior quality as it is cross pollinated, heterozygous and propagated exclusively by seeds. Standardisation of *in vitro* propagation techniques will help for the rapid propagation of elite palms which have been selected for desirable traits.

Progress of work in other centres

Preliminary studies on coconut tissue culture were made by Eeuwens (1976), Pannetier and Buffard Morel (1982) and Branton and Blake (1983). Stray instances of plantlet production *via* somatic embryogenesis were reported by Raju *et al*. (1984) at the CPCRI, Kasaragode and Blake (1988) at the Wye College, London. However, the response from various explants are erratic and the conditions for the morphogenesis are very critical.

Proposed programme

In vitro propagation of coconut *via* direct or callus mediated somatic embryogenesis and organogenesis will be attempted. Standardisation of the culture medium and conditions, explant source and season will be made for obtaining optimum response *in vitro*.

Oil palm

Oil palm is one of the first major tree crop in which the techniques for *in vitro* propagation have been perfected. However, the protocol is not well defined as the techniques are evolved and maintained by private firms.

In vitro cloning of elite palms would be of great significance as conventional vegetative propagation methods are impossible.

Progress of work in other centres

In vitro techniques for the clonal propagation of oil palm have been standardised by Jones (1974), Corley *et al.* (1976) and Rabechault and Martin (1976). Corley *et al.* (1981) observed the progenies to be having uniformity in the rate of leaf production and flowering pattern. However, recent reports have indicated instances of sterility among the progenies.

Proposed programme

In vitro clonal propagation of oil palm will be attempted *via* somatic embryogenesis and organogenesis. *Ex vitro* establishment of the plantlets and long term field evaluation of their performance will also be made.

Ginger

In vitro clonal propagation of ginger will help in the rapid propagation of disease free plants having superior traits. It can reduce the cost of planting material and alleviate the problem of storage of seed rhizomes. Use of such plantlets will help to reduce the errors in the study of the physiology and biochemistry of rhizome formation.

Progress of work in other centres

Successful instances of the *in vitro* propagation of ginger were reported by Hosoki and Sagawa (1977) from Hawaii, Pillai and Kumar (1982) from Jaipur and Bhaghyalakshmi and Singh (1988).

Proposed programme

The programme aims at increasing the *in vitro* multiplication rate of ginger *via* enhanced release of axillary buds. Attempts will also be made for the *in vitro* propagation *via* somatic embryogenesis and organogenesis.

Turmeric

In vitro clonal propagation of turmeric will help in the rapid propagation of disease free plants having superior traits. It can reduce the cost of planting material and alleviate the problem of storage of seed rhizomes. Use of such plantlets will help to reduce the errors in the study of the physiology and biochemistry of rhizome formation.

Progress of work in other centres.

Nadgauda *et al.* (1978) at the NCL, Pune reported the *in vitro* propagation of turmeric using rhizome buds. Shetty *et al.* (1980) at the CPCRI, Kasaragode could obtain callus mediated shoot regeneration from rhizome buds.

Proposed programme

Attempts will be made for increasing the rate of *in vitro* multiplication of turmeric using rhizome buds. *In vitro* propagation *via* somatic organogenesis and somatic embryogenesis will also be tried.

Pepper

In vitro propagation of pepper will help in the rapid multiplication and early establishment of genotypes having superior traits, including resistance to biotic and abiotic stress conditions.

Progress of work in other centres

Mathews and Rao (1984) at BARC, Bombay could induce multiple shoots from pepper seedlings. Callus production could also be induced. However, differentiation was found difficult.

Progress of work in KAU

Rajmohan (1985) could induce organogenesis in the callus of pepper. However the study was handicapped by the systemic presence of bacteria in the explants. Attempts are being made to overcome the problems and to standardise techniques for the successsful *in vitro* cloning of pepper.

Proposed programme

The proposed programme aims at the *in vitro* clonal propagation of pepper using various explants and different routes of *in vitro* propagation.

Selected banana varieties

Njalipoovan is a choice variety of banana grown extensively in the homesteads of Kerala. Considerable intra-clonal variation exists in Njalipoovan which can be exploited through rapid methods of propagation. Another popular variety Red Banana, is a shy suckering one, with characteristic flavour and colour.

Almost all the banana cultivars are affected seriously by "bunchy top" and production of disease-free planting material assumes primary importance.

In vitro propagation techniques have not been standardised for most of the varieties, although commercial *in vitro* production has commenced in a few like Robusta and Nendran. Rapid multiplication of the choice varieties *via* tissue culture can ensure the availability of disease-free planting material for the homesteads of Kerala.

Standardisation of *in vitro* propagation techniques for popular varieties like Njalipoovan, Red Banana, Palayankodan etc. is envisaged under the programme.

Breadfruit

Conventional methods of vegetative propagation of breadfruit offers a low percentage of success and is cumbersome. Standardisation of effective tissue culture techniques will be rewarding for the multiplication of the crop.

Reports of the successful *in vitro* propagation of breadfruit are lacking. At the KAU, preliminary studies were done by Rajmohan (1985) for the culture establishment of various explants. Callus could be induced from immature inflorescence.

Proposed programme

In vitro clonal propagation of breadfruit will be attempted *via* the various routes.

Rare orchids

The Western Ghats harbour a number of rare and endangered orchids. Interest in orchid cultivation is on the increase among Keralites. The proposal aims at the *In vitro* propagation of rare orchids by optimising the culture media and conditions.

Foliage plants

There is a steady increase of internal demand for foliage plants. Several of them are being exported also. Rapid multiplication of foliage plants like miniature crotons, *Ficus spp.*, *Dieffenbachia*, *Aglaonema*, *Phillodendrons*, *Caladium*, *Alocasia*, *Aralia*, Cordyline and *Dracaena* will be of economic significance.

The proposal envisages standardisation of *in vitro* propagation techniques for these plants.

Medicinal plants

In vitro techniques are of great significance for the rapid multiplication of rare and valuable medicinal plants of Kerala. Not much work has been carried out in this respect. The proposed programme aims at developing techniques for the tissue culture propagation of important medicinal plants of Kerala.

Tree species

The potential benefits of establishing forestry plantations with selected vegetatively propagated clonal planting stocks have long been established. However, most of the economically important tree species are not amenable to the conventional methods of vegetative propagation. *In vitro* propagation will be of help in this respect.

Successful micropropagation techniques have been standardised in teak, tamarind, eucalyptus and a few other species. Field evaluations have also been made (Mascarenhas *et al.*, 1987; Gupta and Mascarenhas, 1987).

Proposed programme

Attempts will be made to standardise the technique of micropropagation and field planting of the following tree species which are popularly used for plantations as well as social/agroforestry programmes in Kerala.

Timber species	**Soft wood species**
Tectona grandis	*Ailanthus* species
Silia silocaspa	*Macranda* species
Dalbergia species	
Lgerstromia lansiolata	**Multipurpose species**
	Pterocarpus marsupium

Mango

Rapid vegetative multiplication of selected plants having superior traits and suitable to the agroclimatic conditions of Kerala alone can increase the productivity of this crop in the state. It can also meet the public demand for choice varieties. The present genetic stocks are characterised by inferior quality due to the heterozygosity resulting from seed propagation. Micropropagation can also help to prevent the rare varieties becoming extinct.

Progress of work in other centres

Preliminary studies on the *in vitro* propagation of mango were made by Rao *et al.* (1981) at the IIHR, Bangalore. Litz (1985) in USA could induce large number of somatic embryoids from the nucellar explants of polyembroyonic mango cultivars.

Proposed programme

The proposed programme aims at standardising tissue culture techniques for the clonel propagation of important mango varieties of Kerala. Field establishment of the plantlets and long term evaluation of their performance are also envisaged.

Clove

Clove is an important spice crop of Kerala, earning considerable foreign exchange. Much variability exists regarding the yield, bearing habits and quality of the spice. Effective vegetative propagation methods are lacking. *In vitro* propagation of this crop can be of great importance for producing clonal plants of superior traits, including dwarf tree stature for facilitating easy harvest of the buds.

Successful reports on the *in vitro* propagation of this crops are lacking.

Proposed programme

The project aims at standardising tissue culture techniques for the clonal propagation of clove. Field establishment and evaluation of the plantlets will also be made.

Cinnamon

Cinnamon is another important spice crop of Kerala. *In vitro* propagation can help in the rapid multiplication of selected plants.

Progress of work in other centres

Only preliminary studies have been made for the *in vitro* propagation of cinnamon (Rai and Chandran, 1987).

Proposed programme

The proposal envisages the standardisation of *in vitro* techniques for the clonal propagation of cinnamon. *Ex vitro* establishment of the plantlets and the evaluation of their field performance will also be attempted.

Anthurium

The demand for this costly ornamental plant is on the increase in Kerala. The crop is being propagated by vegetative means; but the rate of multiplication is extremely slow. *In vitro* techniques can be of great significance.

Progress of work in other centres

Standardisation of *in vitro* techniques in *Anthurium* species *via* somatic organogenesis has been made by Pierik (1975), Fersing and Lutz (1977) and Kunisaki (1980).

Proposed programme

Standardisation of techniques for the *in vitro* propagation of *Anthurium* species suitable for Kerala will be made.

Pomegranate

Pomegranate is an ideal crop for homestead cultivation in Kerala. The juice of the fruit is useful for vine production. The fruit has medicinal properties. There is much potential for export too.

Much variability exists regarding fruit yield and quality. Vegetative propagation through cutting, layering or by root suckers is slow and cumbersome. *In vitro* propagation methods will be useful for the rapid propagation of elite types.

There are only preliminary reports on the *in vitro* propagation of pomegranate. Omura *et al*.(1987) reported plantlet formation from leaf explants. Moriguchi *et al*.(1987) could induce adventitious shoot formation from anthers of pomegranate.

Studies will be made for optimising the culture medium and conditions and identifying the explant source for maximum

response *in vitro*.

Minor fruit crops

Minor fruit crops of Kerala like , custard apple, sapota, guova, rose apple and passionfruit are suitable components of homestead farming. The vegetative propagation methods of these crops are slow and cumbersome. *In vitro* propagation can ensure the large scale adoption of these crops to the homesteads of Kerala.

There are only preliminary studies on the *in vitro* propagation of these crops (Gupta *et al*., 1981; Nair *et al*., 1984).

Proposed programme

Standardisation of *in vitro* techniques will be made for increasing the rate of multiplication of the minor fruit crops of Kerala listed below. Varieties/cultivars ideal for Kerala conditions will be used for this purpose. *Ex vitro* establishment of the plantlets and the evaluation of their field performance will also be made.

Custard apple
Rose apple
Guava
Sapota
Passionfruit

2. *In vitro* genetic improvement of crop plants

The potential applications of plant tissue culture for genetic improvement of crop plants are quite amazing. The conventional methods of plant breeding depends on the sexual cycle for the introduction and manipulation of genetic variation. Tissue culture techniques, on the other hand, offer facility for exploiting the somatic phase. They can be of immense significance for evolving desirable genotypes when integrated with the conventional breeding programmes.

The following aspects of *in vitro* genetic improvement will be considered under the proposal.

Exploitation of somaclonal variation

Tissue culture associated variation (somaclonal variation) is very extensive, related to many important traits like pest and

disease resistance, growth, vigour etc. (Larkin and Scowcroft, 1981). Most of the variations have a genetic or epigenetic basis. Somaclonal variation can be utilised for the improvement of specific traits, particularly where they are lacking in the available germplasm.

Somaclonal variants in rice have been selected by several workers like Suenka *et al*., (1982), Oono (1984) and Reddy and Vaidyanathan (1985). Somaclonal variation occurs in high frequency in the callus cultures of solanaceous vegetables and ornamentals (George and Sherrington, 1984).

The proposal aims at evolving somaclonal variation in the following crops.

Crop	Specific objectives
Rice	Selection of somaclonal variants resistant/tolerant to flood and salinity.
Pepper	Selection of somaclonal variants for disease/pest resistance, dwarf stature.
Tomato Chilli Brinjal	Selection of somaclonal variants for disease/pest resistance.
Ornamentals	Selection of somaclonal variants for variation in petal/foliage colour and appearance.

In vitro mutagenesis

Plant cells can be exposed to physical or chemical mutagens for increasing the genetic variability and for selecting desirable mutants. Unlike the whole plant, a very large number of cells can be screened at one time for desirable traits.

In vitro mutagenesis using chemical and physical mutagens will be attempted in pepper, tomato, chilli, brinjal and selected ornamentals.

In vitro pollination/fertilisation and embryo culture/rescue

Prezygotic incompatibility can be overcome by *in vitro* pollination and fertilisation, followed by the culture of fertilized ovules. Embryo culture has been used to overcome *in vitro* inviability of proembryos as well as immature and mature embryos of interspecific/intergeneric hybrids. It can also be

used for the interspecific/intergeneric transfer of chromosomes or chromosomal fragments and can be an easier alternative to the routes offerred by protoplast fusion.

There are several reports in this regard with reference to various crops (Gosal and Bajaj, 1983; Iyer and Govila, 1964).

In Kerala, with regard to several crops, though there exists a good collection of wild and distant relatives, evolution of desirable varieties through conventional methods has not been successful due to the lack of proper development and growth of the hybrid embryos. The rich variability in the wild genotypes can be effectively explcited to evolve better varieties, by adopting in vitro techniques.

In the case of cocoa, outbreeding is promoted by a pollen incompatibility mechanism that is unique in angiosperms. The incompatibility mechanism sets in just before syngamy, posing hindrance to inbreeding and interspecific hybridisation. The use of in vitro pollination and fertilization can be of help. There is no report on in vitro pollination in cocoa. Embryo culture will also be useful to overcome in vivo inviability of pro-embryos and immature embryos. Large scale multiplication of proven hybrids is possible by the in vitro culture of mature embryos and the subsequent multiplication of sterile plants.

In the case of Coffea arabica the spectrum of genetic variability is narrow as it is self pollinated. The variability among the outbred species of coffee can be effectively utilised for the improvement of Coffea arabica with respect to low caffein content, resistance to pests and diseases, uniform ripening of berries, suitability for mechanical harvesting, tolerance to stress conditions etc. Embryo culture techniques will be of much use in this respect.

Seed setting in ginger has not been achieved yet due to the incompatibility mechanisms in operation. In vitro pollination and fertilisation and embryo rescue techniques can be resorted for this purpose which can open the way for hybridization programme in ginger.

In the case of banana, the poor germination of the hybrid seeds can be overcome by embryo culture techniques.

Interspecific hybridisation in tomato can be undertaken for imparting disease resistance, using in vitrotechniques.

In vitro pollination/fertilisation and embryo culture/rescue will be tried in cocoa, coffee, ginger, banana, tomato and orchids.

Haploid culture and production of homodiploids

The haploid germ cells, usually the immature pollens either enclosed in or isolated from the anther can be cultured to produce plants. Haploid plants constitute a very good system to induce, detect and analyse mutants. It provides a quick way by means of diploidisation to produce homozygous lines in a single step and hence assumes immense significance in perennial crop breeding. It also permits the isolation of recessive mutants and to get gene dosage effect.

There are several reports on the haploid culture of rice (Zhang et al., 1983). New varieties of rice have been derived by anther culture, viz. Huayu-1, Xin Xin etc. and released in China indicating the feasibility of this method for rice improvement. Reports have been made on the haploid culture of tree crops and ornamentals. In the case of cocoa regeneration could not be achieved in the haploid callus induced.

The proposal envisages the haploid culture and production of homodiploids in rice, coconut, arecanut, oil palm, mango, banana, cashew, nutmeg, cocoa, papaya and ornamentals.

In the case of cocoa, diploidisation of the spontaneous haploids will be attempted.

Protoplast culture and somatic hybridisation

Protoplasts are useful for somatic hybridisation, electroporation, microinjection and for exploiting somaclonal variation. Somatic hybridisation is useful in overcoming sexual incompatibility between related species. Although wide crosses often produce cytogenetic abnormalities in the hybrid, it is still possible through somatic hybridisation to make crosses wider than that can be made by conventional means. This method enables the transfer of cytoplasmic traits such as male sterility from one plant species to another.

The project aims at the protoplast culture and somatic hybridisation in cereals, vegetables, spices, ornamentals and other tree crops of Kerala.

3. Basic studies involving Molecular biology and biochemistry

Basic studies involving molecular biology and biochemistry will become essential for the rapid progress in some of the areas indicated above. Since there is no work in progress in these lines at the KAU and as there is a dearth of personnel, only a few aspects will be considered at present as indicated below:

Action of defensive genes

A universal recognition-communication system is present in

plants regulating the expression of various types of defensive genes (Ryan *et al.*, 1987). Plant cell wall contains within its polysaccharide structures, informational components that can be released by attacking insects and microorganisms, that subsequently act either directly or idirectly to activate plant genes involved in the production of defensive chemicals. Investigation of such mechanism will be of interest for studying the role of VAM in imparting disease/pest resistance in crop plants (Area 8).

Aspects of nitrogen fixation

There has been considerable emphasis on the genetics and genetic regulation of nitrogen fixing (nif) and nodulation (nod) genes in the Rhizobium component of the symbiosis (Atkins, 1987). These studies have emphasised the events early in the process, specifically those involved in infection and nodule initiation. It is in nodule development, when the plant appears to exercise greater control over the association, that an effective symbiosis is realised. Research emphasis should be targeted towards the genetics and regulation of nitrogen fixation as a plant process. Studies will be initiated on these lines under the proposal.

Juvenile - adult phase change

In vitro propagation of crop plants (Area 1) is very often handicapped by the maturity barrier problem. With the transition from juvenile to mature phase several species exhibit reduced response *in vitro*. Rejuvenation of cells and tissues is known to improve the *in vitro* multiplication efficiency of crop plants (Bonga, 1982). A detailed analysis involving molecular biology is relevant in this context. Studies in this regard has been initiated in various centres abroad. Hackett *et al.* (1988) investigated the molecular difference between juvenile and mature *Hedera helix* plants. Isolation of poly A(+)RNAs, *in vitro* translation and subsequent analysis could detect difference in polypeptides. They have indicated that there is a strong rationale for concluding that maturation ralated differences in phenotypic characteristics are the result of differential gene expression. There are several reports that in the cells mRNAs and proteins act as the rejuvenating signals (Gillham, 1978). The above aspects will be studied in relation to juvenility, maturation and rejuvenation of crop plants.

d. *In vitro* secondary plant metabolite production

The *in vitro* production of secondary plant metabolites which are of pharmaceutical significance has become increasingly important in the field of biotechnology. Cell strains containing amounts of secondary plant metabolites greater than those found in intact plants have been isolated by clonal selection. It is possible to increase the amount of compounds by establishing the most suitable medium for the production of these metabolites. Addition of suitable precursors is also possible for enhancing

the production. There are several reports on the *in vitro* production of secondary plant metabolites (Brain, 1974; Mehta and Staba, 1970; Roller, 1978).

The proposal aims at standardising the techniques for the *in vitro* production of useful secondary plant metabolites from the following plants.

Crop	Metabolite
Mucuna pruriens	L-Dopa
Catharanthus roseus	Vincristine and vinblastin
Rauvolfia serpentina	Serpentine, cymalicine and resirpine
Dioscorea species	Cortico steroids
Vanilla planifolia	Vanillin

5. *In vitro* preservation of germ plasm

In vitro preservation of germ plasm, using cryopreservation and other techniques can ensure the indefinite storage of elite and unique genotypes. It is a potentially reliable means of eliminating the conventional labour intensive maintenance practices, the problem of biotic and abiotic hazards, loss of viability, natural mutation and space. Meristem culture is the most suitable for the purpose since the progeny derived from the *in vitro* culture of meristems shows very little genetic variation. Techniques for the *in vitro* preservation of germ plasm have been standardised for the several crops like cassava, potato, orchids etc. (Kartha, 1983).

Techniques for the *in vitro* preservation of germplasm will be standardised for mango, banana, jack, cocoa, ginger, turmeric and medicinal plants.

6. Genetic modification of microbes

Plasmids of plant pathogenic bacteria

Plasmids have been demonstrated to be responsible for virulence, as in the case of *Agrobacterium tumefacience*.

The main objectives of the proposed study are to find out the presence of plasmids in plant pathogenic bacteria like *Xanthomonas* and *Pseudomonas*, responsible for many diseases of crop plants in Kerala, and to examine whether or not these plasmids are responsible for the expression of virulence and the recently observed resistance to antibiotics. It also envisages the possibility of using plasmid cured strains for the biological control of plant diseases. besides, if plasmids responsible for antibiotic resistance can be identified, it may also become essential to try new combination of antibiotics/chemicals (like

bactrinol) for the control.

Protoplast fusion/mutaion of blue green algae

The identified blue green algae fix nitrogen at neutral and slightly alkaline pH. However, the rice areas of Kerala have generally low pH values. From Pazhanchira (near Attingal) ela a few acid tolerant strains of blue green algae have been identified. But these strains do not fix nitrogen. It is worthwhile to attempt protoplast fusion between the two types for evolving acid tolerant and nitrogen fixing strains of BGA. Mutation, using physical mutagens like UV and chemical mutagens like EMS will also be tried for this purpose.

Hypovirulence in Rhizoctonia solani

It is possible to identify hypovirulent strains of *R. solani* since different grades of virulence exist. Such strains can be used for inducing hypovi.ulence in virulent strains by way of hyphal fusion.

7. Breaking the immune response of insect pests to phathogens

The melonfly, *Dacus cucurbita* Coq., is an agricultural pest, both serious and refractory. There is a vulnerable aspect from the insect's point of view, if properly tackled may lead to successful mangagement of this notorious pest biotechnologically. This is the antibacterial immune system of the fruitflies. Recently Postlethwait *et al.*, (1988) have shown that the system in a related species, *Ceratitis capitata*, is comprised of certain antibacterial proteins. If in some way the inherent defence of the fruitflies against bacteria and wounds can be broken, it will pave way to an efficient and biotechnologically sound way of combating these perennial pests. As the melonfly is a serious problem in Kerala, the project is proposed.

8. Mass multiplication of insect pathogens/beneficial organisms

Mass multiplication of insect pathogens

Microorganisms are efficient tools in the biocontrol of insect pests in Agriculture. But their multiplication is difficult due to the lack of host insects (which are not always available under field conditions). Recent studies have indicated the possibility of artificial culturing of the microbes using tissue culture techniques. There are very effective polyhedrosis viruses (NPV) for the control of noxious pests like *Opisina arenosella*, *Spodoptera litura*, *Spodoptera mauritia*, *Anadevidia peponis*, *Sylepta derogata*, *Pericallia ricini* and *Margaronia indica*. CPV is effective against *Rhynchophorus ferrugineus* and GV

can control Pericallia ricini.

The proposal aims at standardising techniques for the mass multiplication of these viruses using tissue culture techniques.

Mass multiplication of beneficial organisms

Mushrooms

Mushrooms serve as a good source of proteins, vitamins and minerals. Their innate flavour is an additional quality. They have been recommended by FAO as food contributing to the protein nutrition of the developing countries depending largely on cereals. They are indoor crops, utilising organic wastes.

Work on edible mushrooms was started in the KAU during 1977. Paddy straw mushroom (Volvariella spp.) and oyster mushroom (Pleurotus spp.) were identified as suitable for cultivation in Kerala.

The project aims at establishing pure culture of selected species of edible mushrooms and evolving new strains by breeding, mutation and protoplasmic fusion. Standardisation of in vitro techniques for the large scale cultivation of newly evolved strains will also be undertaken.

Azolla - Overcoming the problems associated with the mass production and widespread use

At present there are problems associated with the widespread use of Azolla in paddy fields.

The use of Azolla is labour intensive. Maintenance ponds are required for multiplication of the inoculum for the field and the weight of the fresh material and its fragile nature prevents its long distance transport (Peters and Calvert, 1987).

The use of Azolla spores for an inoculum depends on the increased understanding of the factors which trigger sporulation. Such information is essential for the future utilisation of Azolla.

The proposal aims at the induction of sporulation, standardisation of the method for harvesting the spores and storing them. Study of the factors governing prevention of sporulation is also of interest since sporulation results in diminished nitrogen fixation.

VA mycorrhiza - Use in imparting disease/pest resistance

VA mycorrhiza has been shown to have the ability to impart disease/pest resistance in crop plants, through various means (Hayman, 1987). It can increase the production of polyphenols in

plants, which in turn can increase the disease/pest resistance. VAM inhibited chlamydospore formation by Thielaviopsis basicola on tobacco roots as it increased the arginine and citrulline content (Wilhelm, 1973). Tomato wilt caused by Fusarium oxysporum was also reduced by VAM infection. There are several reports on the adverse effect of VAM on plant parasitic nematodes by the suppression of reproductive process and competition for the same root niches (Sikora and Schonbeck, 1975).

Studies on the VAM have been initiated at the Kerala Agricultural University (Rajendran Pillai, 1989).

The role of VAM in imparting disease/pest resistance in crop plants and the mechanism involved will be investigated under the project. Cases like Meloidogyne infestation of crop plants and Fusarium wilt of tomato will be considered for the purpose. Standardisation of techniques for the large scale production of inoculum and the methods for field inoculation will also be made.

9. Pheromone production

The successful reports on the effective control of sweet potato weevil (Heat,1985) and a few other crop pests have evoked much interest for the use of sex hormone for biological control of pests.

Following the field trials at CTCRI, Sreekaryam using the pheromone Z-3-dodecen-1-ol(E)-2-butenoate for controlling sweet potato weevil, the RRL, Trivandrum could synthesise the chemical at a very low cost of Rs 5/- for one gram. This has given the reputation to India as the fourth country to produce synthetic pheromones.

There is much scope for the biological control of the major pests of crop plants of Kerala, using pheromones. This will necessarily involve the identification, isolation and purification of the pheromones, laboratory and field bioassays and production of synthetic chemicals that are quantitatively and qualitatively indistinguishable from the natural products. Evaluation of the efficacy for the large scale field control of pests is also necessary. The proposal considers research on these lines involving a few major pests and their pheromones.

B. TEACHING

The Kerala Agricultural University, charged with the responsibility or organising teachning programmes relevant to the agricultural sector, proposes to organise post-graduate programmes (M.Sc. and Ph.D. level) aimed at producing agricultural bio-technologists who can be involved in research, extension education and training activities. The Centre would be groomed as an Advanced Centre where visiting scientists can spend a few months to an year for undertaking collaborative research

programmes. These programmes would be organised by the core staff proposed, with the help of co-operating scientists in other departments / projects / schemes of the University.

C. TRAINING AND CONSULTANCY

The state of Kerala has already taken lead in commercialising the results achieved in biotechnology research. M/s A. V. Thomas and Co. and several commercial nurseries are getting involved in rapid multiplication of elite plants and are exporting tissue-cultured plants. The day is not far off when tissue culture facilities would be common in the nurseries. Other areas in biotechnology such as genetic improvement of crop plants, cryopreservation of germplasm, in vitro production of plant metabolites etc. can also be expected to be put to commercial use very soon. The demand for technicians trained in the various aspects of biotechnology work would grow rapidly. The Centre proposes to offer short-term training programmes aimed at generating technical man power suitable for the above mentioned areas of work.

It is also proposed to have exchange of scientific personal with laboratories within and outside the Country. The training component will be for about 30 man months with 15 outside the Coutry. Consultancy is proposed for six man months with three man months for experts from outside the Country.

III. FACILITIES REQUIRED

Infrastructure

A Biotechnology Centre with a plinth area of 1429 sq. m. and a green house complex (1000 sq. m.) are proposed for the main centre at Vellanikkara. A smaller Laboratory facility with 398 sq. m. area and a green house complex (500 sq. m.) are proposed at Vellayani (Plans attached as Annexures I and II).

Equipment

The items of equipment for carrying out the proposed work are listed in Annexure III, with their approximate cost.

Personnel

A core staff (Annexure IV) has been proposed for the main centre and the sub centre as indicated in the introductory. The core staff will be responsible for manning the centres, and for running research, extension education and training programmes. Necessary help in the supporting disciplines would be made available by the other departments of the University.

IV. FINANCIAL ESTIMATE

The financial requirement for a five-year period has been estimted and presented in Annexure V.

UNIT FOR BIOTECHNOLOGY IN ANIMAL SCIENCES

I. INTRODUCTION

Biotechnological approaches have been shown to have tremendous impact on livestock production and health care. It has its application in evolving animals with increased productivity and resistance to diseases, in the production of biologicals with high degree of specificity, in the manipulation of rumen micoflora for improved or new pathways of degradation of food stuff and in the production of substances which directly affect animal physiology. Embryo manipulation, preservation and transfer have now become important tools for genetic improvement of livestock. Investigations on various aspects of genetics, molecular biology, microbiology, ultrastructure , embryotransfer and biochemistry with reference to Animal Sciences are in progress in this university.

To give a proper thrust and to coordinate the various investigations on these frontier areas it is necessary to establish a Centre for Biotechnology for Animal Sciences including Fishery Science at the Mannuthy campus.

II. PROGRAMMES

A. RESEARCH AREAS

1. Analysis and cloning of DNA
2. Vaccine production based on recombinant DNA technology
3. Hybridoma and monoclonal antibody production
4. Gene manipulation of rumen microflora for improved utilisation of nutrients
5. Embryo preservation, manipulation and transfer
6. Production of transgenic fishes for better growth and yield

1. Analysing and cloning of DNA

Restriction enzymes are used to render a DNA preparation into specifically cut collection of fragments, which may then be cloned into a particular site of a suitable vector. In addition to their use in the creation of recombinant DNA molecules, these enzymes have found a vide variety of other applications such as in the mapping of small genomes and segments of DNA, in determining the minimum number of copies of a particular gene present in a genome, in preparing DNAs of a length appropriate for sequencing and in the analysis of DNAs from different individuals for the presence of restriction fragment length polymorphysm of diagnostic importance. These techniques would help in the analysis and manipulation of the genome and its function at a level of precision.

Initially the following technical programme would be undertaken:

A)i. Analysis of R-plasmids and transposons in bacterial organis: E.Coli, Salmonella and Staphylococci pathogenic to poultry and cattle.

ii. Development and selection of cloning vectors like plasmids, cosmids and bacteriophages to be used in vaccine production based on r-DNA technology.

B)i. Construction of genome libraries for selected viral organisms.

ii. Production of diagnostic antigens using genetic engineering technology.

Hybridisation is used to detect genes or portion of genes in the DNA of organisms. The presence of a portion of a gene may be used as an indication that the whole gene is present or even that the whole organism is present. The "Southern blot" allows one to determine the size of the gene fragment on which the portion of the gene occurs. This would help to detect small quantities of infectious agents with the degree of specificity that would allow differentiation between vaccine and virulant strains. If the pathogen can be identified in a rapid, simple test in the farm treatment of control measures can be initiated immediately without waiting for antibody to develop.

C) Restriction fragment length polymorphysm and genetic improvement in Dairy cattle

Restriction fragment length polymorphysms are a new class of genetic markers uncovered by means of recombinant DNA methodologies.

The method is based on the ability of certain enzymes, known as restriction enzymes to cleave DNA at specific sites. The DNA fragment formed can be separated by gel electrophoresis. From the continuous smear of bands so formed specific fragments are detected by use of an appropriate "probe", which consists of closed DNA sequence homologous to a solid support, such as nitrocellulose paper, and exposed to radioactively labelled probe under conditions that promote DNA-DNA hybridisation. The unhybridised radioactivity is washed out and the filter is dried and placed against photographic film for autoradiographic exposure. After film development the specific DNA fragment that hybridised with the probe is visualised as a band on the film.

Base changes and chromosomal abberations alter the sequence that are recognised by restriction enzymes, as a result, the given restriction enzyme will not cut a given DNA molecule at the same point in two individuals. Consequently fragments of different sizes will be found formed and will be located at different positions on the film after autoradiography.

Restriction fragment length polymorphysms have been found at high frequencies in all species studied. This technology has made it possible to search for direct or indirect effect of restriction fragment length polymorphysm on traits of economic importance. Such effects, if detected would be of great utility in breeding programmes. By the use of RFLP it will be possible to rapidly build up a comprehensive genomic map, though it requires a cooperative effort from many laboratories.

2. Vaccine production using r-DNA technology

Recombinant DNA technology is of great help for the production of efficient vaccines for animal protection. The vaccines produced by this technique called either synthetic vaccines or subunit vaccines are more potent and safe than the conventional vaccines. The subunit vaccine production strategy employs the cloning of the major immunogenic gene into a suitable vector system. These are coupled with promoter sequence and part of some other bacterial protein gene so that the inserted antigenic gene is expressed in the product which can be used as vaccines after coupling with proper adjuvants and carriers. The synthetic vaccine approach utilizes the amino acid sequence of the major epitope of the antigenic protein for chemically synthesising the peptide sequences . These small peptides are then combined with a carrier molecule. It will also be possible to improve live virus vaccine by modifying virulence, antigenicity, spread, persistence and other characteristics.

Initially production of sub unit vaccine will be attemted against Duck Virul enteritis and Infectious bursal disease.

Live vaccine production based on deletion mutation technique will be attempted later.

3. Hybridoma and monoclonal antibody

Hybridoma technology has wide application in animal health systems. They are very useful in immunodiagnosis and immunotherapy. Monoclonal antibodies can be used to distinguish granulocytes, macrophages, T & B lymphocytes. Antigens encoded by the major histocompatibility complex are another target for monoclonal antibody production. High affinity monoclonal antibodies can be developed against hormones associated with reproduction. Just as monoclonal antibodies are being used for standard histological techniques in diagnostic pathology, they can also be used to study development of cells of reproductive system. Monoclonal antibodies might also be used for sex determination.

The technical programme envisaged is production of monoclonal antibodies against specific antigens which could be used for tests as agglutination, precipitaion, neutralisation, lysis, antibody dependent cytotoxicity, radio immuno assay and enzyme linked immuno assay.

d. **Gene manipulation of rumen microflora for improved nutrient utilization.**

A potential target for genetic manipulation of microorganisms is the rumen ecosystem. By manipulation of genes controlling specific metabolic pathways it would be possible to reduce the need for dietary protein in ruminants by increasing the level of amino acids by rumen bacteria. Similarly the ability of the rumen system to increase the ability to degrade lactate more efficiently so that the danger of lactic acidosis developing when ruminants are given high grain diets. Transfer of genes between organisms would involve the production of degradative enzymes such as cellulases which would help efficient fibre degradation. It is also possible to modify the amino acid composition of rumen bacterial protein with the objective of reducing the need for dietary protein supplements.

The main objective will be to augment the digestibility and utilisation of crop residues from the farm environment and industrial wastes in cattle and goats either by biostimulation in the rumen or by genetic manipulation of microbes to modify their fermentation characteristics by augmenting lignin degradation, reducing methanogenesis, enhancing cellulolysis and enhancing degradation of toxic factors.

5. **Embryopreservation, manipulation and transfer**

Utility of cows of high genetic potential can be increased many times by increasing calf crop through super ovulation. Artificially produced monozygotic twins are potential specimens for comparative experiments. The transport of frozen embryo over long distances can be inexpensive means of exporting livestock. It accelerates genetic progress by facilitating progeny testing of females and reduces genetic interval. This technique has an exciting future like sexing of embryo. By cloning carbon copies of the best animals can be made and this can radically change the conventional breeding systems in livestock. Intensity of selection is increased when progeny testing in female is made possible.

The programme envisaged are:

i. Propagation of livestock of superior genetic potential by long-term preservation of embryos.

ii. Conservation of native breeds of livestock and endangered species of animals by preserving the embryos.

iii. Embryo splitting to produce identical twins and cloning of embryos to produce valuble research material.

iv. Superovulation, sexing of embryos and *in vitro* ferti- lization.

v. Experimental production of chimeras and transgenic animals for genetic improvement of offsprings.

6. Fishery Science

Studies in fishries employing biotechnology will be taken up in due course.

B. TEACHING AND TRAINING

This centre would gradually develop academic programmes. Initially courses will be offered for the post graduate students in specific areas of biotechnology. Once the infrastructure and staff components are completed it would be possible to offer Masters degree programme in biotechnology in Animal Sciences. Training facilities would also be provided both for professional scientists and paratechnical staff.

III. FFACILITIES REQUIRED

Building

It is very essential to have a separate building for the biotechnology unit to provide all necessary facilities. A building with an area of 1200 sq m is proposed (Annexure V). In addition animal facilities with an area of 500 sq m is also required to maintain experimental animals.

Equipment

The list of equipment required is provided in Annexure VI.

Personnel

It is necessary to have the core staff indicated in the Annexure VII to manage the biotechnology unit.

Details of Training for Scientists

It is necessary to provide short term and long term training for Scientists in established laboratories to get acquianted with latest techniques. For a five year period training would be required for 30 man months. Provision is also necessary.

I Year	:	12 man months	
II Year	:	6 man months	
III Year	:	6 man months	
IV Year	:	3 man months	
V Year	:	3 man months	(15 months outside India)
Total	:	30 man months	

C. CONSULTANCY

Visits by Consulting Scientists from other Institutions	: 6 man months (3 man months - Experts from outside India)

PROPOSED BIOTECHNOLOGY CENTRE (VELLANIKKARA)

Molecular Biology ($36 m^2$) 6 m

Biochemistry ($36 m^2$) 6 m

Genetic engineering ($36 m^2$) 6 m

Fermentation ($30 m^2$) 5 m

corridor ($69 m^2$)

Head of the Centre ($30 m^2$) 6 m, 5 m

Stenographer 4 m

Visitors room

Staff room ($60 m^2$) 10 m, 6 m

Library ($60 m^2$) 6 m

Corridor ($36 m^2$)

Media preparation cum equipment room ($60 m^2$) 10 m

Store ($24 m^2$) 4 m

Vessel cleaning room ($24 m^2$) 4 m

Corridor

Autoclaving ($32 m^2$) 8 m, 4 m

Office ($50 m^2$) 5 m

Seminar Hall ($100 m^2$) 10 m

Lecture Hall ($80 m^2$) 8 m

Lecture Hall ($80 m^2$) 8 m

Corridor ($108 m^2$) 2.5 m, 3 m

Toilets ($24 m^2$) 4 m, 6 m

Disinfection aria ($54 m^2$) 9 m

Inoculation room(s) ($42 m^2$) 7 m

Corridor ($60 m^2$) 3 m

Culture room I ($30 m^2$) 5 m, 6 m

Culture room II ($30 m^2$) 5 m

Culture room III ($30 m^2$) 5 m

Corridor ($18 m^2$) 3 m

Corridor ($54 m^2$) 3 m

Special Exit

Culture room VI ($30 m^2$) 5 m

Culture room VII ($30 m^2$) 5 m

Culture room VIII ($30 m^2$) 5 m

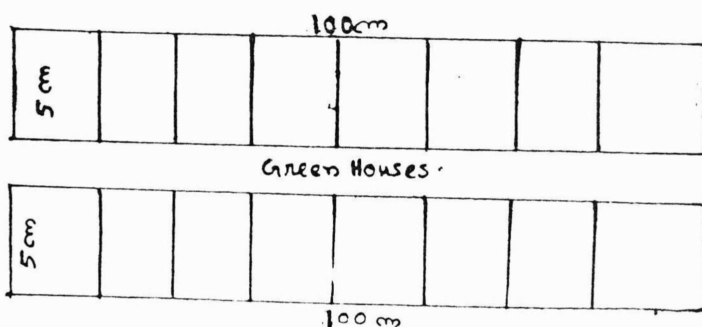
Plantlet delivery room ($18 m^2$)

3 m

Special Exit

Main Building: $1429 m^2$

Green House : $1000 m^2$



PROPOSED BIOTECHNOLOGY CENTRE AT VELLAYANI

18 m
Corridore (36 m²)
2 m

Library (36 m²)
6 m

Media preparation cum scientists' room (60 m²)
6 m
10 m

Seminar Hall (48 m²)
8 m

Corridore (32 m²)

Vessel cleaning room (12 m²)
4 m
3 m

Autoclaving room (12 m²)
3 m

Disinfection area (6 m²)
2 m

Inoculation room (12 m²)
4 m
3 m

Store room (12 m²)
4 m
3 m

Toilets (12 m²)
2 m
6 m

2 m

Area : 398 m²

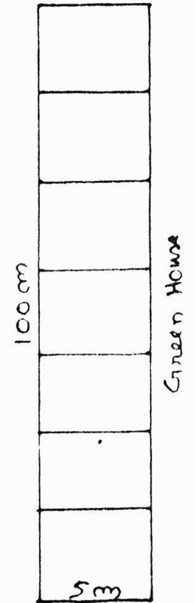
Special Exit

Corridore (30 m²)
2 m

Culture room I (30 m²)
6 m
5 m

Culture room II (30 m²)
5 m

Culture room III (30 m²)
5 m



Annexure III
LIST OF EQUIPMENT

Vellanikkara Centre

Equipment	Qty	Amount (lakhs)	Areas for which essential
Trinocular microscope with phase contrast, flouresence and photography attachments	1	4.0	
Binocular microscope	1	0.5	
Stereo microscope	1	0.5	
Inverted microscope	1	2.0	
Ultra centrifuge (80,000 rpm)	1	8.0	
Freezing system for cryopreservation	1	3.0	
Gas chromatography unit with different detectors	1	3.0	
UV-visible spectrophotometer	1	3.0	
Infra red spectrometer	1	3.0	
Microprocessor controlled incubator-shaker	1	2.0	
Sonicator	1	0.5	
Bioreactor	1	3.0	
Environmental growth chamber	1	3.0	
Deep freeze (upto - 80°C)	1	1.0	
Freeze drier	1	0.5	
Isoelectric focussing system	1	0.5	
Microfraction collector	1	0.5	
Photography unit with Poloroid camera	1	1.5	
Advanced projection systems	-	1.5	
Microfilm reader	1	0.5	
Micropipettes	-	1.0	
Membrane filter system	1	1.0	
Laminar air flow chamber	4	1.5	
Electronic balance	2	1.5	
Microwave oven	2	0.5	
PC, dotmatrix printer & Electronic typewriter	-	1.0	
Autoclave	1	1.0	
Ice flaking machine	1	0.5	
Microprocessor controlled liquid scintillation system	1	4.0	
Generator and UPS	1	2.0	
Air conditioning	-	10.0	
Equipment costing less than Rs 10,000 (pH meter, voltage stabilizers, vacuum pump, magnetic stirrer, lux meter, hot air ovens, slide warming table, paraffin embedding bath, vacuum cleaner, intercom units, fire exstinguishers etc.		2.0	
Total		67.5	

Vellayani Centre

Equipment	Qty	Amount (lakhs)	Areas for which essential
Trinocular microscope with phase contrast, fluorescence and photomicrographic attachments	1	4.0	
Inverted microscope	1	2.0	
Stereo microscope	1	0.5	
Ultra centrifuge (80,000 rpm)	1	8.0	
Sonicator	1	0.5	
Deep freeze (upto - 80°C)	1	1.0	
Photography unit with Poloroid camera	1	1.5	
Membrane filter system	1	1.0	
Laminar air flow chamber	2	0.75	
Electronic balance	1	0.75	
Microwave oven	1	0.5	
Autoclave	1	1.0	
Ice flaking machine	1	0.5	
Generator and UPS	1	2.0	
Micro-processor controlled Incubator-shaker	1	2.0	
Environmental growth chamber	1	3.0	
Isoelectric focussing system	1	0.5	
Microfraction collector	1	0.5	
Advanced projection systems		1.5	
Micropipettes		1.0	
Airconditioning		3.0	
Equipment costing less than Rs. 10,000	-	1.5	
Total		37.0	

Annexure IV
PERSONNEL PROPOSED

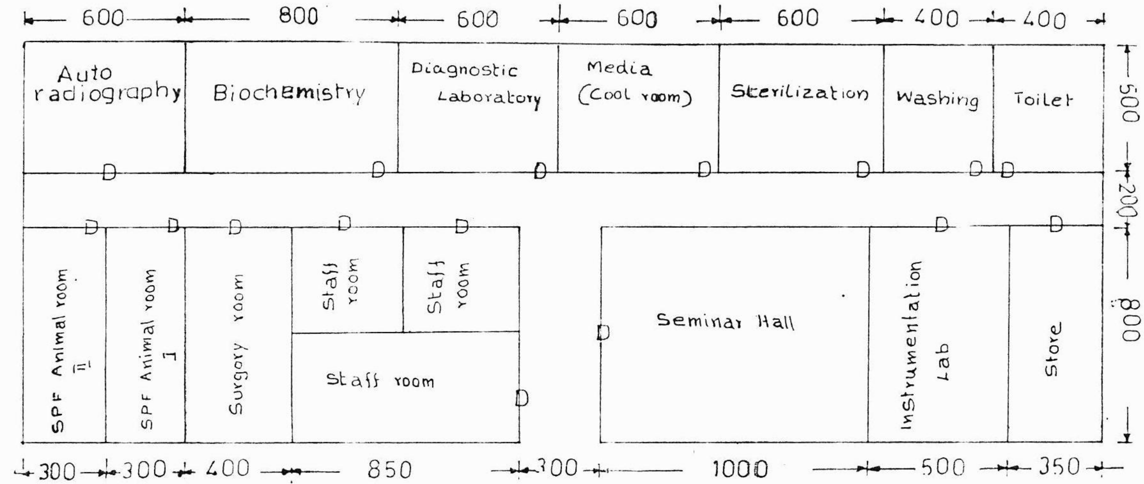
Vellanikkara Centre

Category	No:	py1	py2	py3	py4	py5	Total
Scientists							
Professor							
Biotechnology	1	1	1	1	1	1	1
Assoc. Prof.							
Mol. Biology	1	1	1	1	1	1	1
Biochemistry	1	-	1	1	1	1	1
Asst. Prof.							
Horticulture	1	1	1	1	1	1	1
Genetics	1	-	1	1	1	1	1
Pl. Physiology	1	-	-	1	1	1	1
Supporting staff							
Lab. Asst./ Technician	2	1	2	2	2	2	2
Refrigeration Mechanic (Electrition)	1	1	1	1	1	1	1
Administrative							
Stenographer	1	1	1	1	1	1	1
Asst. Gr.I	1	1	1	1	1	1	1

Vellayani Centre

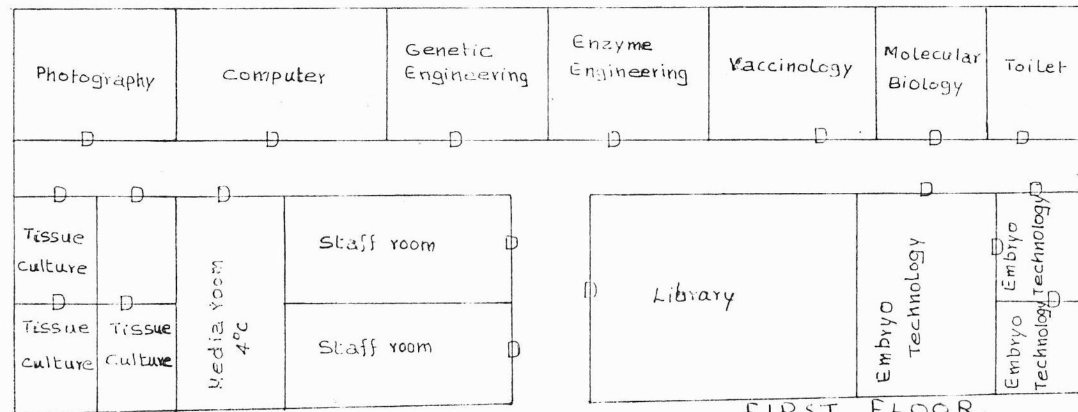
Category	No:	py1	py2	py3	py4	py5	Total
Scientists							
Assoc. Professor							
Horticulture	1	1	1	1	1	1	1
Asst. Professor							
Ag. Botany	1	1	1	1	1	1	1
Horticulture	1	-	1	1	1	1	1
Supporting Staff							
Lab. Asst./ Technician	1	1	1	1	1	1	1
Administrative							
Typist	1	1	1	1	1	1	1
Asst. Gr.II	1	1	1	1	1	1	1

PROPOSED BIOTECHNOLOGY CENTRE MAIN CAMPUS



GROUND FLOOR

PLINTH AREA 1200 Sqm
SCALE 1:20



FIRST FLOOR.

Annexure VI

LIST OF EQUIPMENT

Animal Science Unit

Equipment	Qty	Amount (lks)
Ultra centrifuge with rotors	1	10.0
Superspeed centrifuge	1	6.0
Liquid scintillation counter (PC attached)	1	6.0
Trans illuminator with poloroid camera	1	2.0
Electrophoretic apparutus with power packs	1	3.0
Speed vac	1	1.0
Ultrafiltration assembly	1	2.0
Autoradiography equipment	1	1.0
Ice flaking machine	1	2.0
Deep freezer (-70° C)	1	1.0
Deep freezer (-20° C)	3	0.9
Laminar air flow chamber	2	0.6
Fume hood	1	0.3
Radiation monitor	1	0.2
Refrigerated microfuge	2	4.0
DNA sequencer	1	3.0
Micro pipettes (Ependorf or Hilson)	5 sets	1.0
Disposable plasticware.	(L.S.)	1.0
Milli Q water system	1	1.0
Millipore filter assembly	1	0.5
Orbital shaker	1	1.0
CO_2 incubator	1	2.5
Fermenter	1	0.5
Tissue homogenizer	1	0.3
Tissue sonicator	1	0.2
Precision balance	4	2.5
UV spectrometer	1	2.0
pH meter	2	0.3
Autoclave	2	0.6
Hot air oven	2	0.3
Generator 25 KVA	1	3.5
Refrigerator	3	0.4
Air conditioner	15	4.0
PC with accessories	1	1.0
Electronic typewriter	1	0.3
Reprographic equipment	1	3.0
Inverted microscope	1	2.0
Stereo microscope	1	1.0
Trinocular microscope with attachments for photography, phase contrast, fluorescence	1	4.0
Binocular microscope	1	2.5
Isoelectric focussing unit	1	2.0
Rotary vacuum evaporator	1	1.0
Flow cytometer	1	4.0
HPLC	1	3.0
DNA synthesiser	1	1.0
Photographic unit	1	1.0
Equipment costing less than Rs. 10,000 each	(L.S.)	10.0
Total		99.5

Annexure VII

PERSONNEL PROPOSED

Animal Science Unit

Category	No:	PY1	PY2	PY3	PY4	PY5	Total
Scientists							
Professor	1	1	1	1	1	1	1
Associate Professor	2	1	2	2	2	2	2
Assistant Professor	3	1	2	3	3	3	3
Supporting staff							
Laboratory Technician	3	1	2	3	3	3	3
Laboratory Attender	3	1	2	3	3	3	3
Administrative staff							
Office Asst. / Typist	2	2	2	2	2	2	2

Annexure VIII
ESTIMATE OF EXPENDITURE

Agricultural Biotechnology - Vellanikkara Unit

Item	PY1	PY2	PY3	PY4	PY5	Total
Infrastructure	46.0	-	-	-	-	46.0
Equipment	67.5	-	-	-	-	67.5
Chemicals	3.0	1.0	1.0	1.0	1.0	7.0
Glassware	2.5	1.0	0.5	0.5	0.5	5.0
Furniture	4.0	0.5	0.5	-	-	5.0
Books and Journals	1.0	1.0	1.0	1.0	1.0	5.0
Training and TA	0.5	0.5	0.5	0.5	0.5	2.5
Maintenance	1.0	1.0	1.0	1.0	1.0	5.0
Other items	0.5	0.5	0.5	0.5	0.5	2.5
Man power	1.9	3.41	4.56	5.46	6.50	21.83
Total	127.9	8.91	9.56	9.96	11.00	165.33

Agricultural Biotechnology - Vellayani Unit

Item	PY1	PY2	PY3	PY4	PY5	Total
Infrastructure	15.0	-	-	-	-	15.0
Equipment	37.0	-	-	-	-	37.0
Chemicals	1.75	0.75	0.5	0.5	0.5	4.0
Glassware	1.5	0.5	0.5	0.25	0.25	3.0
Furniture	2.0	0.5	0.5	-	-	3.0
Books and Journals	0.5	0.5	0.5	0.5	0.5	2.5
Training and TA	0.5	0.5	0.5	0.5	0.5	2.5
Maintenance	0.5	0.5	0.5	0.5	0.5	2.5
Other items	0.5	0.5	0.5	0.5	0.5	2.5
Man power	1.22	1.87	2.26	2.72	3.25	11.32
Total	60.47	5.62	5.76	5.47	6.00	83.32

Animal Science Unit

Item	PY1	PY2	PY3	PY4	PY5	Total
Non Recurring						
Buildings & fittings	20.00	10.00	-	-	-	30.00
Furniture	-	5.00	-	-	-	5.00
Equipment	40.00	50.00	10.00	-	-	100.00
Recurring						
Chemicals & glassware	10.00	8.00	5.00	5.00	5.00	33.00
Books & Journals	2.00	1.00	1.00	1.00	1.00	6.00
Other Contingencies	3.00	3.00	3.00	3.00	3.00	15.00
Pay & Allowances	4.50	6.00	6.25	6.50	6.75	30.00
Training, Consultancy, T.A.	2.00	2.00	1.00	1.00	1.00	7.00
.Total						226.00

ABSTRACT OF ESTIMATED EXPENDITURE

Centre for Biotechnology in Agriculture and Animal Sciences

Item	Rs. in Lakhs
Infrastructure (Building, fittings and furniture)	104.00
Equipment	204.50
Chemicals and Glassware	52.00
Books & Journals	13.50
Pay & Allowances	63.15
Training & Consultancy	32.00
Other items & Contingencies	27.50
Total	496.65

UNIT FOR BIOTECHNOLOGY IN AGRICULTURE	270.65
UNIT FOR BIOTECHNOLOGY IN ANIMAL SCIENCES	226.00
TOTAL	496.65

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