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DESIGN REPORT
ON
TISSUE CULTURE AND BIOTECHNOLOGY OF HORTICULTURE CROPS

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SUMMARY

The design team visited five research centers, including the Indian Institute for Horticultural Research, The Central Plantation Crops Institute, The National Center for Cashew Research, the Dr. Y.S. Parmar University of Horticulture and Forestry and the Indian Agricultural Research Institute. Each of these institutions had been identified by the Indian Council of Agricultural Research as a center for an expanded research program in tissue culture and biotechnology of horticulture crops. The primary objectives were to determine the capabilities of each institute to carry out a larger research program in these areas. We were introduced to ongoing research programs in tissue culture and reviewed the facilities at each institute. On this basis, we have made recommendations 1) strengthening and 2) expanding the research components, 3) upgrading laboratory equipment and facilities, 4) training in specific areas and 5) identification of key international scientists whose collaboration during a hypothetical five year project would be critical for achieving the stated goals of a greatly expanded program.

The identified crops include: mango, citrus, apple, banana, coconut, cashew, oil palm, cacao, walnut, gladiolus, rose, tomato and chillies. The areas that were identified that need strengthening include:

A. Micropropagation, including shoot tip culture and related techniques for virus elimination, de novo regeneration and field acclimatization.

B. Production of advanced breeding lines through embryo rescue, production of dihaploid plants and somatic hybridization.

C. Somatic cell genetics, based upon the development of efficient morphogenic systems, the recovery of useful somaclonal variants and the development of useful molecular markers.

D. Genetic engineering, including all aspects of research involving recombinant DNA and field evaluation according to accepted US/NIH/APHIS and government of India Department of Biotechnology guidelines.

The success of a project of this nature can only be realized within active multidisciplinary groups. Transfer of the technology to the grower is an equally important ingredient of the research, and the investment in biotechnology would be a failure if this did not occur. It is critical that collaborating institutions should be involved in the later stages of any project to assure commercialization of the technology.

I. INTRODUCTION

India has a rich diversity of agro-climates suitable for growing of a large variety of horticultural and plantation crops. The country ranks second in the world after China in vegetable production and third in fruit production. However, the full potential of these crops has not been realized so far because of pre-occupation of the planners and workers with attaining self-sufficiency in foodgrain crops, which has now been achieved. The potential of horticulture crops to ameliorate the poverty of farmers and to improve the overall economic standards of the country has been recognized. Concerted efforts are currently underway for diversifying agriculture from the age-old cereal crop-based farming system to horticulture-based land use particularly in arid, semi-arid and hilly areas. This trend has, therefore, greatly increased demand for planting material of improved varieties of these crops, which in many cases cannot be made through conventional propagation techniques. Micropropagation is an alternative technique for the major commercial crops. In some crops, the technique has already been standardized, but has not been adopted on commercial scale. In other crops, such as mango, cashew, coconut, etc. this technique has not been developed for important varieties.

Despite the diversity of available cultivars and amount of ongoing research, the productivity of most of the crops falls well below the desired levels. Consequently, the total production of fruits and vegetables in particular that is available in the country falls well below the demand for a growing population. This situation is going to be further aggravated by rising population and shrinking land resource. Consequently there is a need to improve yield of these crops using both conventional and advanced breeding approaches to overcome major production constraints. The use of biotechnology for crop improvement would be one of the major avenues available to achieve the desired goal.

During the past few decades great progress has been made in the basic understanding of plant cell morphogenesis. Initially, the main emphasis was to understand the in vitro responses of plant cells and tissues. These studies indicated that cultured plant cells are capable of producing a whole plants in a suitable in vitro environment. It is now apparent that potentially unlimited numbers of plants can be obtained mainly via three regeneration pathways, i.e., 1) axillary budding of the shoot primordia; 2) somatic embryogenesis, and 3) organogenesis. Since the first regeneration pathway preserves the integrity of the meristem structure the progeny have been generally observed to be true-to-type. Theoretically, every plant species can be regenerated; however, it has now become apparent there are unique and variable factors that must be understood for each species to be cultured successfully. For example, a plant species that has been regenerated in one laboratory may be recalcitrant in another. Some

cultivars have been more difficult to regenerate than others. Most model regeneration systems have been described in Solanaceous species. However, most horticulturally important species have been found to be considerably more difficult to regenerate than species within that group. It is, therefore, imperative that efforts should be focused on horticultural species belonging to other plant families particularly the woody fruit and nut crop species. Moreover, identification and or isolation of desirable diversity in horticultural crops can be achieved using *in vitro* techniques, e.g., somaclonal variation, interspecific hybridization using embryo rescue and protoplast fusion and modification of single gene traits using recombinant DNA techniques.

A. CURRENT STATUS OF MICROPROPAGATION OF HORTICULTURAL CROPS

1. India

From personal discussions with various scientists working on plant tissue culture in India, it is possible that a much larger effort has been made to develop regeneration protocols than has actually been published. There are some reports on the *in vitro* multiplication of fruit tree crops, e.g., walnut (Cheema and Mehra, 1982), date palm (Sharma et al., 1986), coconut palm (Iyer, 1981), papaya (Rajeevan and Pandey, 1986), citrus (Rangaswami and Maheshwery, 1958), apple and pear (Mehta and coworkers, see literature), pineapple (Mathews et al., 1982), sugar apple (Nair et al., 1982), guava (Amin and Jaiswal, 1988), mulberry (Bapat and Rao, 1984) and jackfruit (Jaiswal and Amin, 1992). There have also been a few published reports of *in vitro* propagation of ornamental species, e.g., carnation (Choudhry and Rajan, 1990), rose (Choudhry, 1990), bougainvillea (Doreswami, 1991) and orchids (Singh, 1991). There has been some effort to commercialize micropropagation. Indo-American Hybrids (Bangalore) and A.V. Thomas (Cochin) are currently selling various ornamentals, bananas and cardamon. Tata Energy Research Inst., Spic India, Unicorn Biotech., and Hindustan Levers Ltd., have now established commercial units for micropropagating commercially important plants. However, there are several crop species i.e., mango, walnut, coconut, cashew, cacao, apple, citrus, etc., for which commercial micropropagation technology has yet to be developed.

2. Outside India

There are many reports of plant regeneration in several species of horticultural importance to India, e.g., cashew (Ninan et al, 1983), coconut (Pannetier and Buffard-Morel, 1982; Branton and Blake, 1983), oil palm (Jones et al., 1982), apple (Zimmerman and Broome, 1980), date palm (Tisserat, 1981), cacao (Pence et al, 1980; 1981), banana (Ma and Shii, 1977), mango (Litz, 1984; Litz et al., 1982), strawberry (Boxus, 1971), grapes (Krul and Myerson, 1980); peach (Hammerschlag, 1980), pear (Singha, 1980), papaya (Litz and Conover, 1977), citrus (Rangan et al., 1968; Navarro and

Murashige, 1977), carnation (Ziv 1987), rose (Hasegawa and coworkers), etc. From some of these reports it has been difficult to determine the success of field trials of regenerated plants. However, the complete technology from test tube to field has been described for banana, papaya, citrus, apple, walnut, grape, peach, date palm, oil palm several types of berries, rose, carnation, gladioli etc. Efficient plant recovery still needs to be developed for coconut, cashew, cacao, mango, etc.

B. VIRUS INDEXING OF HORTICULTURAL AND AGRONOMIC CROPS

In vitro procedures have also been used to produce disease indexed plants with isolated meristems and nucelli utilized as explants. Kartha (1987) has reviewed the progress in this field. Using meristem tip culture often in conjunction with thermotherapy viruses have been eliminated from ginger, banana, strawberry, apple, Prunus species, rhubarb, potato, sweet potato, cassava, pellargoniums, lillies, gladiolus, narcissus, carnations, chrysanthemum, tulips, etc. In vitro micrografting has also been used to eliminate viruses in citrus, peach and apple. Quite often plants regenerated through organogenesis from the dark areas from leaves displaying mosaic symptoms are also virus free (Murakishi and Carlson, 1976; White, 1982 and Dean, 1982). Addition of antiviral compounds such as Virazole to plant growth medium has also resulted in tissue free of the infecting virus. Virus indexed plants produced in vitro are now routinely used for upgrading plantings of many horticultural species and for international exchange of germplasm.

C. GENETIC TRANSFORMATION OF HORTICULTURAL CROPS

Following the earlier demonstration of transformation in model experimental systems, i.e., tobacco and tomato, transgenic plants expressing marker genes have now been obtained in a few horticulturally important crop species, i.e., apple (James et al., 1989), walnut (McGranahan et al., 1988), strawberry (James et al., 1990; Nehra et al., 1990), cranberry (Serres and McCown, 1991), papaya (Fitch et al., 1990), mango (Mathews et al., 1992), peach (Smigocki and Hammerschlag, 1991), citrus (Vardi et al., 1990), grapevine (Mullins et al., 1990) and plum (Mante et al., 1991). Horticulturally important traits, e.g., pest tolerance conferred by the expression of the *Bacillus thuringiensis* encoded toxins have been introduced in to tomato (Fischhoff et al., 1987), apple and walnut (Dandekar et al., 1989; 1991), virus tolerance in tomato (Beachy and coworkers) and papaya (Gonsalves personal communication) through the expression of the coat protein of ToMV (tomato ringspot virus), PRV (papaya ringspot virus) and the control of tomato fruit ripening through the expression of antisense mRNA for the enzymes polygalacturonase (Sheehy et al., 1988; Grierson and coworkers), ACC synthase (Theologides and coworkers) and the expression of the bacterially encoded enzyme ACC deaminase (Klee personal communication). Other horticulturally

important traits that are currently under investigation include genes that would enhance, cold tolerance, desiccation tolerance, tolerance to fungal infections, male sterility, plant architecture and form, etc.

D. RATIONALE OF PROPOSED PROJECT

One of the major purposes of this project is to encourage the development of infrastructural and manpower capabilities in India in the field of tissue culture for horticultural crops. It is hoped that this project would facilitate the integration of biotechnology with ongoing programs in the areas of cultivar improvement and development. It is also anticipated that this integration will ultimately lead to transfer of technology and advanced germplasm to the private sector. At various institutions in India horticulturists and breeders have identified/developed superior selections of many ornamental and fruit crop species. However, they are unable to release this material for public distribution due to shortage of plants. Conventional methods of clonal propagation are ineffective for meeting the initial demand. In some cases, clonal propagation using conventional techniques is infeasible, e.g., coconut. In these circumstances micropropagation would ensure a constant supply of elite plant material.

The same infrastructure and expertise could be used to develop plant tissue culture methods for cultivar improvement by somatic cell genetics and recombinant DNA technology. It is possible that fruit crop production would be expanded to include marginal areas with the development of plants improved in their capacity to withstand biotic and abiotic stress conditions. The recovery of stress tolerant selections can be accomplished through in vitro mutagenesis or identification of somaclonal variants and by incorporating specific genes through recombinant DNA technology. Useful traits like water /temperature stress tolerance, disease/pest resistance present in germplasm of related and unrelated wild species can be introgressed into elite cultivars with the development of embryo rescue techniques (vegetable crops) and somatic hybridization (stress tolerant rootstocks for fruit trees).

E. TARGET CROP SPECIES

The following horticultural crop species have been identified for the sub-project on the application of tissue culture and biotechnology:

1. Fruit crops: Mango, apple, banana, citrus, walnut
2. Plantation crops: coconut, cashewnut, cacao, oil palm
3. Vegetables: tomato, chillies
4. Ornamentals : rose, gladioli

These are commercially among the most important horticultural crops of India. Attempts have been made in several laboratories in India to use tissue culture methods for mass propagation and for genetic improvement of these species. In all these crops (except vegetables) there has been short supply of the quality planting material to meet the rising demand for new plantings. This trend is likely to continue if supplies are not increased.

In case of tomato and chillies, quality hybrid seed can be produced efficiently utilizing conventional technologies. However, genetic engineering techniques could be used for transferring desirable horticultural traits into current grown varieties.

1. Fruit Crops

a. Mango

The Mango, because of its great utility, occupies a pre-eminent position amongst the fruit crops grown in India. No other fruit, except banana, is so closely associated with the history of agriculture and civilization as is the mango. Records suggest that it has been in cultivation in the Indian sub-continent for well over 4000 years. In India, it is connected with all phases of life from birth to death.

In terms of area (1063 510 h) and production (9337520 tonne) mango occupies first rank among the fruit crops in India. Fully 65% of the world's mangoes are produced by India. The mango is the world's fifth most important fruit. Although about a thousand varieties of mango are known to exist in India, an ideal mango variety is still lacking. Most of the current varieties were selected as chance seedlings for characters like fruit size, fruit quality and precocity, dwarfness, prolificity and regularity of bearing. Self-fruitfulness and resistance to pests and diseases remained unselected. These characters are of vital importance for making the best use of our shrinking land resources, reducing the cost of cultivation and for improving the productivity per unit area. Combining all the desirable characters in a single variety through conventional breeding methods is difficult, if not impossible, since the mango is a heterozygous crop of suspected allopolyploid origin. However, it can be made easier through tissue culture.

In vitro methods could be utilized for rapid and efficient propagation of rootstocks and of dwarf selections in which there is an acute shortage of scion material. Moreover, the application of recombinant DNA technologies to mango are dependent upon the availability of efficient de novo regeneration pathways. The following objectives should be addressed:

- i Micropropagation through somatic embryogenesis from nucellus and meristem tissue culture techniques.

- ii Identification of marker genes and regenerate transgenic plants by applying sophisticated techniques of genetic engineering to incorporate genes of interest.

b. Apple

Apple is a major temperate fruit crop of northern India, where contemporary European and North American varieties of apple have been grown for more than 100 years. Apple is more important than mango worldwide, and is the most popular temperate fruit in India. The industry is considered to be progressive; however, there are acute problems involving disease, e.g., apple mosaic, in many scion cultivars, and a shortage of both scion and rootstock cultivars. In order to address this situation, a programme for the production of virus-indexed nuclear stock of the important varieties is essential. This would be coupled with the establishment of an advanced disease diagnostic laboratory. The shortage of plant material could be addressed through the development of efficient micropropagation methods.

c. Banana

Banana is among the most important fruits of India, and has an important center of diversity in the northeast. The crop is grown throughout the country except in the far north. Despite its national importance, and despite the fact that its worldwide production exceeds all other fruit, banana is recalcitrant from a conventional breeding standpoint; it is a sterile triploid. All modern cultivars have been derived via somatic mutation from ancestral plants. There are many serious diseases of banana in India, including bunchy top (virus), cucumber mosaic virus (CMV), Panama disease (*Fusarium oxysporensis*), etc. There is a need to produce disease-free nuclear stock of banana and accurate diagnostic tools and lines with high levels of existence. Breeding objectives can only be met through the use of plant cell culture techniques.

d. Citrus

Citrus is among the major fruit crops of India. In India, citrus ranks 3rd after mango and banana in area (244040 h) and production (1952240 tonnes). Citrus fruit production in the country is about 9% of the total fruit production. Mostly mandarin, sweet orange and limes are under commercial cultivation.

Commercial citrus cultivation started in India around 1900 and by 1920 decline became a major problem, particularly in poorly drained soil in Maharashtra. In 1956 I.C.A.R. opened three centres to study citrus dieback: Gonicoppal in Karnataka, Shrirampur in Maharashtra and I.A.R.I. in Delhi. The main cause of citrus decline in India is 'Dieback' and is of complex nature. Besides involving various pathogens, soil disorders including nutritional

deficiencies. rootstocks and cultural practices are responsible for causing this disorder.

Citrus trees are generally propagated by grafting buds of a scion cultivar onto seedling rootstocks, which allows the transmission of virus and mycoplasma diseases. However, virus elimination could be possible through micrografting and/or regeneration via nucellar and somatic embryogenesis. Rapid clonal multiplication of desired rootstock would boost the establishment of high density orchards, which is the vitally important. Development of rootstocks tolerant to salt and drought stresses is also considered to be important in certain regions of the country. Research objectives include:

- i. Production of virus free citrus plants by adopting micrografting technique.
- ii. Screening and selection of biotic and abiotic stress tolerant germplasm in vitro through somatic embryogenesis from nucellar embryo suspension cultures.
- iii. Genetic manipulation for developing disease and stress tolerant and dwarf plants through genetic engineering.

e. Walnut

Walnut is currently not widely grown in northern India; however, it is known that this region is close to the centre of major genetic diversity. Scattered seedling trees occur throughout the north; however, commercial exploitation of this crop has been impeded by the lack of effective vegetative propagation methods for this crop. Although considerable effort has been directed toward this goal, it is still impractical under Indian conditions. Micropropagation has been demonstrated to be effective with this crop in the U.S.A. Similarly, genetic transformation has been used to address serious insect pest problems with this crop. In order for walnut to become important in India, the development of appropriate technologies is essential.

2. Plantation Crops

a. Coconut

India is one of the largest coconut producing countries of the world. The country ranks third after Philippines and Indonesia. The annual production of coconut oil in 1987 was estimated at 2.13,000 tons, accounting for 7 per cent of the total vegetable oil production in the country (United Coconut Association of Philippines, 1988). Coconut makes a significant contribution to the national income. The average value of production of the crop at the current price level is around Rs.9,000 million and export earnings around Rs.322 million, mainly through the export of coir

and coir products (Govt. of India. 1987).

The annual planting material requirement of coconut in the country has been estimated at 15 million seedlings. Although India was the first country to produce a coconut hybrid more than 50 years ago, it has not been possible to meet the growing total requirement of hybrid seedlings. For effective clonal multiplication of hybrids and elite palms, tissue culture is a prerequisite to meet the entire requirements of planting materials.

Coconut is presently grown in an estimated area of 151 million ha producing about 9283 million nuts per annum. Kerala produces nearly 4394 million nuts in an area of 0.876 million ha. This contributes to 58.02% and 47.3% of area and production respectively.

Major problems impeding enhancement of production and quality

- i. The coconut is a cross pollinated palm grown in many tropical countries for nut and oil.
- ii. Coconut has a long juvenile phase.
- iii. Coconut exhibits very high heterozygosity and it is exclusively seed propagated.
- iv. Conventional breeding methods for crop improvement is time consuming and difficult.
- v. Coconut is grown in our country in small holdings, mostly of less than a hectare in size and hence small farmers and landless labourers are the major participants in coconut production. Per capita availability of coconut in India is very low (8 nuts/year) as compared to per capita consumption in countries like the Philippines (190 nuts/year) and Sri Lanka (165 nuts/year).
- vi. One of the major yield depressing maladies in Kerala is root (wilt) disease caused by Mycoplasma-like-organisms (MLOs). The annual loss due to this disease is estimated to be 968 million nuts. Apart from this, other diseases like Ganoderma and Tatipakka are prevalent in Kerala, Tamil Nadu and Andhra Pradesh and there are no effective control measures.
- vii. Most of the coconut cultivation is under rainfed conditions. Hence the crop is subjected to moistures stress, resulting in very low yields.
- viii. There is a lack of superior quality seedlings to meet the requirements of coconut growers. The demand for coconut seedlings in the country is about 15 millions annually

as against the production of 1 million seedlings which has resulted in a large gap between supply and demand.

b. Cashew

Cashew is probably the most versatile of all the nuts and is widely used by the confectionery industry. A large percentage of proteins with all essential amino acids and easily digestible fats, make the cashew a highly nutritive part of the diet. India holds a monopoly in the supply of cashew kernels in the international market. The crop covers 530,900 ha. of which 29% is in the state Kerala. Disproportionate to this area figure, Kerala's contribution of production of nuts is considerably higher (49%), and thus is an important cash crop of the state.

Cashew is a highly cross pollinated crop and hence seed propagation often gives a highly heterogenous population with less productivity. Though there are a few high yielding selections like Vengurla-2 (43 kg/tree/year) and exceptional yielders with more than 100 kg/tree/year, the national average yield of cashew is only 2.1 kg/tree/year. The low productive orchards are to be replanted with high yielders, and the demand for qualitative cashew seedlings is increasing. Vegetative propagation through veneer grafting, budding, air-layering, and epicotyl grafting cannot meet the demand for superior planting material because of the low rate of multiplication and field establishment. Tissue culture techniques could provide large quantities of clonal planting materials of high yielding hybrids and selections.

c. Cacao

Cacao (*Theobroma cacao* L.) has gained considerable importance in the national economy in recent years due to increasing demand for cocoa products which include chocolate, cocoa oil and cocoa butter. These products find uses in perfumery, preparation of cosmetics, pharmaceutical products and in the manufacture of soft drinks.

In India, cacao is cultivated in a total area of 16,500 ha. mainly in Kerala and Karnataka, producing more than 6,000 tonnes/annum, although several other potential areas for its cultivation have been identified in the states of the North Eastern region.

One of the important constraints in the varietal improvement of cocoa is the lack of genetic variability in the available germplasm. Cocoa germplasm has been obtained from Kew Gardens, England and planted at the Lalbagh Gardens, Bangalore, and these are being utilized for the production of hybrids at our center. Like many other preminals, cacao breeding is both long term and expensive, taking a minimum of ten years for a single generation. It has also been recognized that most of the seedling progenies of

high yielding trees tend to be inferior to their parents and so selections are propagated vegetatively. Raising of clones as planting material would simplify breeding programmes as elite/superior yielding plants could be vegetatively propagated for largescale use. Very few countries have sufficient stock material for making commercial plantings of clonal material produced from high-yielding hybrid varieties. Hence, propagation through shoot tip culture as well as somatic embryogenesis would help greatly in supplementing conventional methods of vegetative propagation.

d. Oil Palm

Although oil palm has not figured as an important component of domestic edible oil production, there has been a recent initiative to establish large plantings in various designated areas of the country. High yielding, perhaps dwarf selections of *pisiferaxdura*, could make a significant impact on meeting the needs of the domestic economy. Cell and tissue culture approaches are the only viable procedures that are available for clonally propagating elite oil palm selections. Work has been underway for many years to describe regeneration protocols via somatic embryogenesis from seedling leaves.

3. Vegetable Crops

a. Tomato and Chillies

Tomato and chillies are among the most important components of the Indian diet. Although both crop species were introduced into India, they have been become naturalized here. Important breeding programmes, shaped and coordinated by IARC have been established that will develop new varieties and breeding lines that are suitable for all parts of the country. Year round production of crops can be accomplished by growing these crops in the "off season" in areas like Himachal Pradesh and Kashmir. It will be necessary to develop active breeding programs in different regions to address location specific production problems.

4. Ornamental Crops

a. Rose

Rose is among the top three cut flowers in international trade. The excellent keeping quality of the flower, variation in form and the ability to withstand long distance transportation has favoured them to become important cut flowers. The Netherlands and Colombia are the leading exporters of cut flowers. India lags far behind other countries in developing a national and export market for ornamental plants. This is aggravated by underdeveloped transportation infrastructure and the lack of elite germplasm to meet stringent international standards. Since India is endowed

with how professional and labour costs, this country could also enter the international cut flower market in the same manner as Kenya, Colombia, Costa Rica and Ecuador. Micropropagation of roses has become standard practice throughout the industry.

Despite the considerable achievement of classical breeding of roses, there remain certain constraints. Chief among these is the limited gene pool, e.g., no plant species possesses the genetic capacity to produce cultivars in a full spectrum of colours. Breeders are also unable to alter horticultural traits in a direct manner. Good production characteristics are dependent on the interaction of multiple gene loci. Therefore, when 2 plants are crossed, new genetic combinations are created that may alter flower colour and other important traits. Mutation breeding, which attempts to alter single traits, has been of limited use. Recent advances in molecular biology, especially in recombinant DNA technology, make it possible to alter plants by adding to their commercial value in a directed fashion without altering their overall production characteristics. This has great potential in cut flower improvement.

b. Gladiolus

Gladiolus, together with rose and carnation, is one of the major ornamental crops of the world. In India, gladiolus is considered to be the most important cut flower. However, very little breeding effort has been devoted to producing cultivars specific for the Indian market and for export. Diseases, particularly viruses, are a problem. There is great demand for higher quality disease-free plants, with a long vase life, and greater variety of color. A biotechnology approach for improving this crop is appropriate.

II. STRATEGY: COMPONENTS OF THE STUDY AND OBJECTIVES

The following components are proposed for the project under consideration:

A. MICROPROPAGATION, REGENERATION AND FIELD EVALUATION OF DESIRABLE GENETIC STOCKS

The plants identified for this project represent a diverse group of horticultural crops that include both herbaceous annual species and woody perennial trees. Regeneration protocols must be developed that can address breeding goals and efficient and faithful propagation and preservation of elite plant materials and for distribution to growers. Horticultural field crop breeding would benefit from the micropropagation of selected parents in F1 hybrid seed programs. The mass cultivation and rapid development of new improved varieties has also underscored the need to preserve newly developed germplasm. Therefore, the simultaneous development of efficient methods for faithful regeneration would permit the

transfer of this technology to NBPGR (National Board for Plant Genetic Resources) for medium and long term storage. Appropriate micropropagation strategies for rootstock/scion cultivars of fruit trees and for production of nuclear disease indexed stock of specific ornamental cultivars and fruit trees should be developed. Since many of the designated woody crop species that are included in this project have been identified as being recalcitrant or difficult to regenerate, appropriate technology transfer and/or development is essential. The specific objectives in plant cell and tissue culture include the development of the following protocols including:

1. Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials, e.g., mango, citrus, banana, grape, cashew, cacao, apple, walnut, gladiolus and rose.
2. Meristem culture and micrografting for the production of virus indexed plants, e.g., citrus, gladiolus, rose, banana, apple.
3. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials, e.g., mango, citrus, rose, banana, coconut, cashew, cacao, oil palm, apple, walnut, tomatoes and chillies.
4. Acclimatization, field introduction and evaluation of regenerated plants, i.e., all species under this study.

B. ADVANCING BREEDING LINES THROUGH EMBRYO RESCUE AND PROTOPLAST FUSION INVOLVING DISTANTLY RELATED SPECIES AND THE RECOVERY OF HOMOZYGOUS DIHAPLOIDS

In many instances the hybridization between distantly related species results in the formation of nonviable seeds. Therefore many horticulturally important traits cannot be introgressed into commercial cultivars. In such cases, embryo rescue techniques have been used to save the F1 progeny. This technique involves the culture of whole ovaries, ovules or excised immature embryos. This procedure has been particularly useful in horticultural field crops like tomato, early ripening fruit crops like peach, the rescue of seedless grape varieties and the rescue of embryos from aborted mango fruit. However, there are instances where the formation of a zygote does not occur after wide interspecific crosses. In such cases somatic hybridization involving controlled protoplast fusion can be utilized. Unfortunately the product of such fusions is an amphitetraploid and therefore cannot be used for scion or cultivar development. However, these somatic hybrids are very valuable in rootstock development. Another useful technique is the recovery of haploid plants from cultured anthers and/or ovules. These haploid plants can be diploidized to produce homozygous parents for a hybrid seed program. This would ensure a highly uniform F1 generation. In order to accomplish this component the following objectives would be carried out:

1. Embryo rescue for the recovery of advanced genetic materials. i.e., mango, banana, grape, tomato, chillies and coconut.
2. Recovery of haploid plants from the culture of anther and ovarian tissues. i.e., tomato, chillies and cacao.
3. Protoplast isolation, fusion, culture and regeneration, i.e., citrus, banana, mango, tomato and chillies.

C. SOMATIC CELL GENETICS

An important prerequisite for the development of somatic cell genetics approaches for cultivar improvement is the availability of a highly reliable and efficient regeneration system from callus or suspension cultures. In vitro culture cycles, particularly involving a prolonged callus phase, can result in substantial genetic variation. While this has been viewed as an undesirable phenomenon in the context of micropropagation, it can however be utilized to select discrete genetic alterations in clonally propagated plants. It has thereby been possible to obtain disease resistant variants in sugarcane and potato and larger petiole size in celery. Among vegetatively propagated fruit crops with long juvenile periods the recovery of somaclonal variants having greater disease or stress tolerance would be a highly significant achievement. In some cases it would be possible to exert selection pressure by the inclusion of a phytotoxin in the culture medium. Surviving cells and their regenerants should also be resistant to the pathogen producing the phytotoxin.

It is often difficult to determine and verify clonal fidelity in plants regenerated from cells and tissue cultures. This is particularly true for woody perennials that can take upto 20 years to reach a stage of maturity for evaluation. The development of markers to provide an accurate fingerprint for a particular cultivar is therefore necessary. It could be possible to verify clonal fidelity in very small propagules using DNA and protein markers. Furthermore, a detailed genetic analysis of these markers would provide a means to predict the alteration of important traits. The development of poly and monoclonal antibodies against particular plant pathogens would provide an efficient diagnostic tool for disease indexing, and would permit the early movement of plant material into the NBPGR system and to the growers.

The following objectives will be accomplished under this component:

1. Highly efficient morphogenetic suspension and cell cultures. i.e., somatic embryogenesis or organogenesis, i.e., mango, banana, citrus, coconut, cashew, cacao, oilpalm, apple, walnut, tomatoes, chillies, gladiolus and rose.

2. In vitro and ex vitro screening for spontaneous and induced somaclonal variants, i.e.. mango, banana, citrus, coconut, cashew, oilpalm, apple, walnut, gladiolus, rose, tomatoes and chillies.
3. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens, i.e.. mango, banana, citrus, coconut, cashew, oilpalm, apple, walnut, gladiolus, rose, tomatoes and chillies.

D. GENETIC ENGINEERING

Recent developments in the application of recombinant DNA technology to plants has resulted not only in the availability of a number of highly efficient vectors but also in the methods to detect, isolate and modify plant genes. For most dicotyledonous plant species the *Agrobacterium*-mediated gene transfer system is suitable as these plants are the natural hosts for this organism. Nontumorigenic (disarmed) strains of the bacteria that have been engineered to include genes from different sources have been developed. A large number of plant species have been transformed with selectable and scorable marker genes including, kanamycin phosphotransferase (APH(3')II), beta-glucuronidase (GUS), nopaline synthetase (NOS) etc. In the case of monocots and other species recalcitrant to *Agrobacterium* infection alternative gene transfer techniques including electroporation, DNA coated microprojectiles can be used. As mentioned above there are a number of horticulturally important genes including genes that affect fruit ripening and postharvest handling quality. e.g., expression of antisense mRNA for the enzymes polygalacturonase (Sheehy et.al., 1988; Grierson and coworkers), ACC synthase (Theologies and coworkers) and the expression of the bacterially encoded enzyme ACC deaminase (Klee personal communication). Pest tolerance conferred by the expression of the *Bacillus thuringiensis* encoded protein toxins have been introduced in to tomato (Fischhoff et.al., 1987), apple and walnut (Dandekar et.al., 1989; 1991). Tolerance to plant virus has been achieved in tomato (Beachy and coworkers) and papaya (Gonsalves personal communication) through the expression of the coat protein of ToMV (tomato ringspot virus), PRV (papaya ringspot virus) respectively.

An important prerequisite for the application of this technology is the development of highly efficient regeneration systems in the target plant species. It is also important that cooperators be identified who have the appropriate skills in the areas of microbial genetics and recombinant DNA technology. In addition that facilities, both laboratory and field, and the routine monitoring procedures should conform to the US/NIH/APHIS guidelines and regulations and the government of India Department of Biotechnology guidelines. It is assumed that the first crop species that will be transformed in this project would be tomato and chillie. Both are Solanaceous species, easy to regenerate, and

have been genetically in several laboratories. Experience with these species should expedite similar transformation studies with other species in this proposal. The following objectives are critical for this component:

1. Acquisition, amplification, analysis and verification of plasmid vectors and microbial strains, i.e., tomato, chillies, mango and rose.
2. Agrobacterium-mediated gene transfer and the selection and identification of transformed cultures and regenerants, i.e., tomato, chillies, mango and rose.
3. Molecular analysis of regenerants to confirm successful gene transfer and the expression of introduced genes, i.e., tomato, chillies, mango and rose.
4. Field evaluation of transgenic plant materials following the US/NIH/APHIS and government of India Department of Biotechnology guidelines and regulations. i.e., tomato, chillies, mango and rose.

E. TECHNOLOGY TRANSFER AND COMMERCIALIZATION

The "green revolution" culminated 100 years of the application of Mendelian genetics to crop improvement. For the most part, staple food crops were targeted and many of the advances remained in the public domain, i.e., public universities, government and international research institutions. It is anticipated that the "biotechnology revolution" will be rapidly exploited by the commercial sector. This will be particularly true for perennial crop species that have a high intrinsic value and that are vegetatively propagated. It is imperative that key alliances be made at early stages of project development with appropriate commercial interests. Many of the gene transfer technologies and horticulturally important cloned genes have already been protected by international patents. It is also possible that there will be innovations during the course of this project that should receive patent protection. These are the realities of biotechnology that must be respected. Suitable policy decisions need to be made in anticipation of the implementation of this project. The following objectives for this component are stated below.

1. Identify key industries and grower groups who will be appropriate recipients of the above technologies, i.e., mango, citrus, banana, apple, walnut, coconut, cashew, cacao, oilpalm, gladiolus, rose, tomatoes, and chillies.
2. Development of research agreements and the protection of intellectual property rights, i.e., mango, rose, tomato and chillies.
3. Pilot testing of new technologies in collaboration with private and or public agencies, i.e., mango, citrus, banana, apple, walnut, coconut, cashew, cacao, oilpalm, gladiolus, rose, tomatoes, and chillies.

III. PARTICIPATING INSTITUTIONS: CURRENT RESEARCH

The mentioned components of research would be carried out at the centers listed in Table 1.

Table 1. Participating research institutions and targeted crop species.

Institute	Crops
Indian Agricultural Research Institute, New Delhi	mango, citrus, chillies, tomato, rose, gladiolus
Indian Institute of Horticultural Research, Bangalore	mango, banana, citrus, tomato, chillies, gladiolus
Central Plantation Crops Research Institute, Kasargod, Kerala	coconut, cacao oilpalm
National Center for Cashew Research, Puttur	cashew
Dr. Y.S.Parmar University of Horticulture & Forestry, Solan.	apple, walnut, tomato, chillies

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

1. Division of Fruits and Horticultural Technology

a. Current Research Activities

i. Mango

Studies on micropropagation of mango are in progress. The major hurdle in micropropagation of mango by shoot tip or meristem tip culture is to control rapid activation of oxidative enzymes after explanting. It leads to the eventual death of excised cultured tissues. The oxidative browning of tissues and culture medium could be eliminated when the shoot tip explants from mango seedlings were grown in a growth chamber under diffused light and cultured in liquid MS medium supplemented with NAA 10 μ m and BAP 0.5 μ m. Although browning of tissues and the medium in such cultures was eliminated, tissues died after 7 weeks.

ii. Citrus

There are no activities that are currently addressing regeneration or micrografting of Citrus species.

iii. Other Fruit Species

Earlier studies focused on developing shoot tip culture as a method for micropropagating female (dioecious) papaya selections. This technology has been transferred to the Division of Biotechnology within I.A.R.I. Other activities have included the development of a micropropagation system for grape cultivars and to utilize embryo rescue when seedless female types are used in hybridization schemes. Neither papaya nor grape falls within the terms of reference of this subproject.

b. Personnel

Research Scientist	(1)	Dr. H.C. Sharma
Technical Assistant		1

c. Facilities

The tissue culture facility consists of a laboratory suite with 2 rooms: one room containing a laminar flow hood and an adjacent room that is utilized as a temperature controlled growth room. Neither room has sufficient space for an expanded programme, and the level of standards is not equivalent to other laboratories that have been included as participating centres in this Indo/USAID subproject. This facility is below nationally and internationally recognized standards in terms of space, convenience and hygiene. A general purpose laboratory across the hallway provides additional access to equipment; however, it is equipped as a general plant physiology laboratory. Modern plant cell and tissue culture facilities should be comparable to microbiology laboratories, and should be relatively antiseptic. Walls in this area were covered by fungal growth where plumbing had leaked from the upper floors of the building.

Equipment and other facilities include:

- Laminar Air Flow
- Autoclave
- BOD Incubator
- Automatic Temperature Controller
- Air Conditioners
- Culture Trolleys fitted with Fluorescent light
- Spectrophotometer with Autoscan
- Deep Fridge
- Refrigerator
- Shaker horizontal
- High Speed Refrigerated Centrifuge

Field space and the use of environmentally controlled chambers and greenhouses. are generally available for this programme by prior arrangement with the Division of Horticulture.

2. Division of Floriculture and Landscaping

a. Current Research Activities

The tissue culture programme of the Division of Floriculture and Landscaping was begun in 1983. Later, in 1985, a research project was formulated with the objective of "Improvement of ornamental plants with the use of plant biotechnology". Three crop species were designated as part of this project. i.e., rose, carnation and gladiolus. Work was initiated with carnation and rose.

i. Carnation

Although carnation does not fall within the terms of reference of this subproject, a substantial amount of work has already been accomplished that has addressed various aspects of morphogenesis. Multiple shoot cultures have been established, and particularly attention was paid to physiological and anatomical events that occur during the process of acclimatization. Differentiation was also reported from leaf and petal callus of carnation, and regenerated plants have been transferred to the field. Preliminary studies directed toward the production of transgenic carnation plants are underway, using *Agro-bacterium rhizogenes* as a vector.

ii. Rose

A rapid micropropagation system has been developed for rose by culturing shoot tips on medium with 0.1 mg/liter NAA and 2.5 mg/liter BA. The maximum number of proliferated shoots that developed during each 4 week subculture period was approximately 6. Rooting has been achieved by subculturing shoots onto medium with 0.5 mg/liter NAA, and plants have been successfully established soil after acclimatization.

Various parameters that can influence explant establishment have been studied, including orientation of the explant and medium composition. The enhancement of in vitro rooting has been found to be dependent on lowering of the total nitrogen in the basal medium to 12.5% of the original nitrogen composition of the culture medium.

iii. Gladiolus

Currently, there is no activity with this species; however, it is included in the divisional work plan at a later date.

b. Personnel

Research Scientists	(4)	Dr. Brijender Singh Dr. S.R. Dohare Dr. M.L. Choudhary Dr. A.P. Singh
Technical Assistants	3	
Laboratory Assistants	2	

c. Facilities

The existing tissue culture laboratory in the Division of Floriculture and Landscaping consists of a media preparation area, a small office and a room that functions both as a controlled temperature growth room and for carrying out sterile transfer activities. There is one laminar flow hood. The space is barely adequate to support the existing programme of research. Currently available equipment include the following:

Laminar flow hood	1
top pan balance	1
analytical balance	1
pH meter	1
water bath	1
refrigerator	1
single distillation	1
shaker	1
air conditioners	2
oven	1

B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

a. Current Research Activities

This institution contains well established laboratories that have been in existence for the past decade and have developed successful protocols for the micropropagation of banana, bougainvillea, rose, and grape. This work has been documented through publication in international journals. In addition over the past two years they have initiated embryogenic cultures from clonal selections of mango; however, this work is still preliminary. As indicated above in Table 1 this institute will focus on, mango, banana, grape, citrus, tomato, chillies and gladiolus. A group of 7 scientists will participate directly in this project, three have ongoing projects in the areas of cell and tissue culture and the remaining four have ongoing programs in the area of cell and

molecular biology. The group has a broad experience with a range of regeneration pathways in both woody and herbaceous plants. In addition some new projects have been initiated in the cell and molecular area, i.e., biochemistry and genetic analysis of plant cells in culture, molecular basis of disease resistance, characterization of bioinsecticides from Bt. The program development in these areas of tissue culture have been focused with respect to goals pertinent to the areas of cell and tissue culture but have yet to be integrated to ongoing breeding and genetic improvement programs at this institute.

The group at this institute has been active for over a decade have worked on banana, mango, grape, pineapple, gladiolus and bougainvillea. Given below is a cropwise discussion of their achievements

1. Banana

They have been able to successfully demonstrate the feasibility of rapidly bulking up propagule material of elite cultivars utilizing micropropagation pathways that involve the shoot tip and the floral apex. Microcloning has been achieved in embryo cultures of banana involving a cross between *Musa acuminata* and *Musa balbisiana*. Hybrid plants obtained from culture have been introduced into the field but now need to be further evaluated for their horticultural attributes. These projects are the most advanced where they have been able to introduce the tissue cultured plants into field. This program needs to be integrated more closely with the commercial sector and with ongoing breeding programs so that important disease problems like, bunchy top and Panama wilt can be effectively addressed. There is also a need to develop techniques for the early diagnosis of these important diseases and for virus detection to identify virus free materials. The group has just begun exploratory work on generating bunchy-top free and Panama-wilt free banana plantlets. Double stranded RNA species has been purified from bunchy-top plants, this RNA could not be detected in healthy plants. This aspect of research needs to be further intensified to develop more precise diagnostic tools.

2. Mango

One of the serious problems that have been observed in the mango breeding program is the abscission of the majority (sometimes as high as 98%) of immature fruit. Using standard embryo rescue techniques they have been able to recover seedlings from newly abscised fruit. These seedlings could then be evaluated in the breeding program. The mango breeder, Dr. C.P.A. Iyer, has been interested in the rescue of 'Neelum' x 'Mulgoa' hybrids.

There is an urgent need for the development of efficient propagation methods for new improved rootstocks and rapid release of dwarf mango cultivars. An approach utilizing the regeneration

of somatic embryos from cultured nucellus tissues of standard cultivars including 'langra', has been employed. Somatic embryos have not developed to maturity at this time, and problems with alternate bearing have slowed progress in this field. This is a difficult regeneration pathway, but they have overcome the most problematic hurdle of achieving embryogenic cultures.

3. Grape

The major objective in grape micropropagation was stated to be the rapid release of salt tolerant rootstock 'Dogridge'. They have been successful in adapting standard grape micro propagation techniques. Again here there is a need for integration with the commercial sector to identify growing areas in India where such salt tolerant rootstocks would be useful. A program for field testing these materials should be developed to verify the utility of this rootstock to sustain grape production in marginal areas.

4. Citrus

No work is currently occurring at this location, however, tissue culture techniques have been extensively reported in the literature and this group certainly has the expertise to make rapid progress.

5. Tomato and chillies

The institute has strong breeding programs on these crops and a close cooperation with the plant breeders will need to occur. Initial exploratory work has been carried out to accomplish embryo rescue in tomato and capsicum. This work will need to be intensified to capture discreet traits from wide crosses. Work has also been initiated on the anther culture of capsicum to generate haploids. In addition at the cell and molecular level leaf mesophyll protoplasts have been successfully isolated and purified from tomato and chillie. A valuable screen for disease tolerance has been developed for tomato. A toxic principle has been isolated from the culture filtrate of *Alternaria solani* which causes wilting and necrosis of tomato genotypes susceptible to the disease. Resistant genotypes showed differential sensitivity to the toxic principle. This approach need to be combined with cell culture program so that both somaclonal and induced variants can be selected to provide disease resistant tomato plants.

6. Gladiolus

Strategies have been developed for rapid and bulk propagation of gladiolus. This program needs to be closely coordinated with the industry and breeding programs to identify key varieties that are needed to be multiplied. It is not clear if this linkage has been made. The group certainly has the expertise to make significant advances with this crop species. However focus will be the key here

and the future directions identified through consultation with the breeding group and recipient industry.

b. Personnel

Given below is a list of the research scientists identified for this project that have a strong experience in the area of plant cell and tissue culture:

Dr. R. Dore Swamy
Dr. Leela Sahiiram
Dr. J.B. Mythili

The below mention research scientists have experience in the area of cell and molecular biology:

Dr. T.V. Ananthanarayanan
Dr. Lalitha Anand
Dr. K.S. Mohan
Dr. Pious Thomas

c. Facilities

Currently the group working on biotechnology is scattered through the main building at IIHR. These consist of individual laboratories that are well-equipped and functional. The following facilities have been made available, three laminar flow hoods, sterilizer, media dispensing unit, air curtains, vacuum cleaner, microtome unit, AO stereomicroscope, UV transilluminator, orbital shaker incubator for bacterial cultures, an LKB mini cold lab complete with fraction collectors, peristaltic pumps for protein purification, lyophilizer, UV spectrophotometer, multitemperature bath, spectrodensitometer, ultra low temperature freezer (Queue) and incubation room for tissue cultures.

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)

a. Current Research Activities

i. Coconut

The coconut tissue culture research programme was established at CPCRI in 1977. Its mandate was to develop a micropropagation system for this species that could be utilized for clonal propagation of elite germplasm.

The first approach tried was to transform the undifferentiated floral primordia to vegetative shoots (bulbils) through in vitro culture of young inflorescence. Culture of rachillae from inflorescences borne at the axil of the third leaf from the spindle on Y3 medium gave shoot-like structures. Of the three types of structures obtained, one was of an indeterminate type that

continued to produce scale leaves. Rooting of these structures was very erratic. Work on seedling leaf tissue culture started during 1982 produced clonal plantlets through direct embryogenesis.

In addition to vegetative propagation of coconut palm, embryo culture was taken up to facilitate germplasm collection and exchange through embryos rather than through the bulky nuts.

Salient achievements of the tissue culture laboratory

1978 Shoot like structure observed from floral primordia of coconut.

1979 Coconut embryos germinated in vitro.

1980 Callus induced in young stem and leaf base tissues from one year old WCT coconut seedlings.

1982 Nodular callus produced from cut ends of leaf explants showed rhizogenesis.

Callus was produced from endosperm of tender coconuts.

1983 Somatic embryos were produced from young leaf explants without a callus phase.

1984 Coconut plantlets were produced from young leaf explants of 2 year old WCT seedlings through direct somatic embryogenesis.

1985 Coconut plantlets established in soil.

ii. Oil Palm

Studies accomplished several years ago at CPCRI demonstrated that somatic embryogenesis could be induced from zygotic embryos and seedling tissues of the oil palm. Plantlets were regenerated, and a few plants were successfully transferred to soil. There has been no attempt since then to work with elite tree selections, although this is the important mandate of the original project.

b. Personnel

Scientific Staff	(3)	Dr. R.D. Iyer Dr. S. Shivashankar Dr. A. Karan
Technical Staff	2	
Support Staff	2	
Steno/Typist	1	

c. Facilities

The laboratories that have been developed for studies on cell and tissue of coconut, oil palm and cacao at the CPCRI provide sufficient space for current studies and the projected expansion of this programme as anticipated in this report. In addition to rooms dedicated to 1) medium preparation, 2) sterile transfer of tissue cultures, 3) air-conditioned, temperature monitored growth room and 4) office. An additional laboratory area is being developed that would accommodate expanded research activities in the area of 5) biochemistry and more 6) air-conditioned growth room space. The tissue culture unit shares the use of an autoclave. The entire facilities were very clean and met with accepted world standards for laboratory hygiene.

The research center is fortunate to have one of the world's largest collections of coconut germplasm, that includes at least 86 exotic and 46 indigenous selections. A very active coconut breeding programme is housed at the center, and is an acknowledged world leader in the production of advanced plant material, but particularly hybrids between tall and dwarf types. Thus, there is a rich and diverse coconut germplasm to support a cell and tissue culture programme at this center.

Ancillary equipment that is essential and available to the coconut tissue culture programme includes:

	No.
Autoclave	1
Millipore water purification system	1
Microscopes	2
Laminar flow hood	3
Rotary Flask shaker	1
Diesel power Generator (100 KVA)	1
Afcoset electric balance	1
pH meter	1
Owalabor top-loading balance	1
BOD incubator	1
Hot air oven	1
Freezer	1
Green house	1
Coconut seedling nursery	1

D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)

a. Current Research Activities

i. Cashew

The tissue culture programme at NRCC has only recently been established. Studies in the make-shift laboratory have involved

the culture of shoot tips and nodal segments from adult trees. Problems associated with polyphenol oxidase activity in the explants have been addressed by incorporating activated charcoal, cysteine or citric acid/ascorbic acid in the medium. It is evident that results are still of a preliminary nature, and include the effect of different phytohormones, particularly 2,4-D, on callus formation and the formation of adventitious roots from explants on medium containing NAA. Limited shoot elongation and axillary bud proliferation have been observed. Stock plants were field-grown trees.

b. Personnel

Scientific Staff	(1)	Mr. Thimmappaiah (cytogenetics)
Technical Staff	2	

c. Facilities

Although the existing tissue culture laboratory is rather primitive, and is housed in its entirety within a single small room, a new research centre has just been inaugurated that will provide considerably more space. The new tissue culture unit will consist of 2 suites of laboratories, each consisting of 2 contiguous rooms. One such pair will be used for media preparation and for accommodating laminar flow hoods. Across the hall, there will be a general purpose laboratory with a connecting door to a plant growth room. At this time, these facilities are empty, except for laboratory benches. It should be possible to modify these rooms in such a way to bring them up to international standards of cleanliness and utility without major modification.

Equipment that has already been either purchased or ordered includes the following:

Laminar flow cabinet (Klenzoids)	1
Top pan balance (Owa) for macroweights	1
Binocular microscope-Nikon	1
pH meter (Elico)	1
Deioniser	1
Double distillation set (Quartz)	1
Refrigerator 286 litre	1
Cooling incubator (Remi)	1
Autoclave-vertical	1
Portable Autoclave	1
Laboratory Incubator	1
Tissue culture trolikes with light and timer	2
Voltage stabilizers	4
Air conditioner (1 ton capacity)	1
Autoclave (Horizontal)	1
Magnetic stirrer	1
Rotary shaker	1
Oven	1

Vacuum pump	1
Bottle washing machine	1
Table top centrifuge	1
Thermohygrograph	1
Lux meter (digital)	1
Vacuum cleaner	1
Millipore filters	1
Automatic pipette washer	1
L.P.G. connection with stove, cylinder and regulator	1
Air conditioner	1
Deep freezer	1
Humidifier with humidistat	1
Dehumidifier	1
Microtome and its accessories	1

E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY

a. Current Research Activities

The Dr. Y.S. Parmar University is located about 15 km from the town of Solan in the hills of Himachal Pradesh. Solan is on the way to the more well recognized town of Simla. This is a small university that specializes in forestry and horticulture and for the purposes of this proposal would be the centre for research on temperate fruit and nut species. At this location the proposed project will be coordinated by the Department of Biotechnology. The department of biotechnology was established fairly recently in 1987 with the aim of providing the leadership and expertise in the area of biotechnology for both the colleges of horticulture and forestry. The department of biotechnology is located in an independent structure in the college of horticulture. The mission of the department of biotechnology includes, development of micropropagation technologies for fruit/nut, forestry, ornamental and vegetable crop species, development of diagnostic tools for virus detection and virus indexing, selection of genetic variants in culture using somatic embryogenesis and somaclonal variation and cell mutagenesis for stress tolerant plants, development of microbial and mycorrhizae with improved capacity to colonize and fix nitrogen on different tree species.

Current research activities center around three funded projects:

i.) A department of biotechnology project that involves the development of micropropagation technologies for *Alnus* and *Quercus* with the aim of developing protocols for the large scale multiplication of these forest tree species. iiA) state funded project that will develop virus free apple plants. iii.) World Bank project on the micropropagation of bamboo. It is anticipated that TERI (Tata Energy Research Institute) and H.P. State

authorities will be the recipients of this technology and be the organization that will do the large scale micropropagation and distribution of plant materials. Given below is a more detailed description of the current research activities in the crops of this proposed project.

1. Apple

This is an important crop in Himachal Pradesh and in temperate regions of India. Over the past two decades there has been a steady increase in the plantings and in apple production. The viral diseases like yellow apple mosaic and chlorotic leaf spot are the most significant. Although a bud wood certification program was initiated in 1983, the recovery of virus free materials is the major problem. Part of the problem stems from the fact that the viruses have not been properly characterized and most of the analysis has relied on symptom expression or graft transmission. This is obviously a very slow process and as a result very little progress has been made in generating virus clean stock. The incidence of visible infection is as high as 20% in orchards. Fortunately there are pronounced differences in susceptibility to the virus among different cultivars of apple. Therefore, there has been some respite from this problem. However, it is clear that a virus indexing system needs to be developed rapidly. This involves developing key viral diagnostic tools that involve both detection of the virus using antisera, DNA probes or PCR and the detection of symptoms using the traditional patch grafts. Attempts have been made to use antisera obtained from East Malling and the isolation of virus from apple or through infection of herbaceous hosts with limited success. A multidisciplinary effort is required to accomplish this objective, where there is a close interaction with plant pathology, immunology, biochemistry, horticulture etc. The group in the department of biotechnology appears to be working somewhat independently, the program should be integrated with other programs and strong centres, e.g., advanced center for virus research in IARI Delhi. ELISA for the detection of virus is routine for a number of apple viruses in the USA and in many parts of the world and therefore, there is no need to re-invent the wheel. This program needs better integration and training of the scientists involved.

2. Walnut

This is an upcoming crop there appears to be a lot of commercial interest, however there is no planting materials. The nursery propagation is still at very early stages of development and at this time they are evaluating different scion materials and grafting methods on seedling *Juglans regia* rootstock. No work has yet been initiated on micropropagation through tissue culture. It appears that a lot has yet to be learned from the field evaluation of locally available of the nursery propagated materials and this has yet to occur. We did not see any field studies on walnut,

therefore we can conclude that this crop is at very early stages of development.

3. Tomato and chillies

These are important crops in the Himachal Pradesh region because of their elevation and unique microclimate, these plants can be successfully grown in 'off-season' with respect to the rest of the country. Therefore, the price structure of these commodities is an attractive feature for local growers apart from the obvious benefit of year-round availability of vegetable commodities for Indian consumers. There is a strong breeding and evaluation program in the vegetable crops department at this facility, however, there are no direct linkages to the department of biotechnology through joint programs. In the biotechnology department they have an active project where they are selecting for water stress tolerant varieties of tomato using a local variety, 'Solan Gola'. This is being accomplished through the selection of callus on media containing different concentrations of PEG with the aim of regenerating stress tolerant plants. It is not clear if water stress is a significant problem in this area. The approach is unlikely to be successful because it is known that the water stress tolerance trait in tomato is a multigenic trait encoded for by at least three loci mapping to three different chromosomes in tomato. Tomato is one of the model experimental systems with a number of wild species, where many useful traits have already been identified and introgressed into advanced breeding lines. A more realistic approach would be to develop an RFLP or marker assisted approach to breed new varieties of tomato, such a program will also foster a close relationship between biotechnology and plant genetic programs in the vegetable crops department.

There are currently no programs in the biotechnology department on chillies. However, there is an active breeding, selection and evaluation program in the vegetable crops department.

c. Facilities

The department of biotechnology is located in an independent building in the court yard of the horticulture building. The facilities consist of a media preparation room that contains an autoclave, single pan balance, automatic media dispensing machine. Adjacent to this is a general biochemistry laboratory that contains UV spectrophotometer, BOD incubator, low speed centrifuge and electrophoresis equipment. The ultracentrifuge is in a faculty office located across the hall from the biochemistry laboratory. At the end of the hallway and adjacent to the biochemistry laboratory is a growth room that is temperature controlled with a window unit, has shelves with lights to grow plants and has a laminar flow hood. A glass house with climate control has been recently built and this is located near the department of biotechnology. Temperature and dust control is accomplished with

window unit airconditioners therefore. dust and temperature control will be some of the problems in critical areas. Also there does not seem to be enough space for an expanded program.

b. Personnel

Research Scientists (6)	Dr. D.R. Sharma Dr. S.V. Bhardwaj Dr. O.P. Sehgal Dr. D.K. Srivastava Dr. K. Khosla Dr. M. Kapil
Field Assistant (1)	Mr. M. Lal

IV. PARTICIPATING INSTITUTIONS: RECOMMENDATIONS

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

Although advanced programmes in plant cell and tissue culture and in molecular biology exist at the NBPGR and in the Division of Biotechnology on the campus of IARI. there seems to be little interaction between them and the Horticulture Divisions. This is surprising. as both of the advanced centers are working with horticultural crops. Similarly, the Center for Advanced Virology does not seem to have any form of collaboration with the tissue culture programmes in the Horticulture Divisions. It is of the utmost importance that interaction should exist; otherwise, the tissue culture programmes in Horticulture will be an end in themselves. and will be unable to address real problems due to the lack of scientific input.

1. Division of Fruits and Horticultural Technology

a. Mango and Citrus Tissue Culture

It is difficult to comment on the ability of the Division of Fruits and Horticultural Technology to play a key role in this subproject. The current facilities are totally inadequate (see above) for an expanded programme involving mango and Citrus with tissue culture and molecular biology components. It would be impossible to carry out the proposed studies in molecular biology and transformation in light of stringent regulations concerning laboratory cleanliness and security. Moreover, the programme of research for mango and Citrus is very ambitious, and would need inputs from more than one research scientist. I would strongly urge that the proposed research should focus on:

- i. regeneration of mango from shoot tips and via somatic embryogenesis from nucellar cultures. Use of this technology in micropropagating elite dwarf cultivars and rootstocks.

- ii. micrografting of Citrus for the production of disease-induced scions. Create the institutional basis for distribution of "clean" material to the industry. Establishing a strong liaison with a plant virologist is essential.
- iii. somatic embryogenesis from nucellar cultures of Citrus. This would lay the basis for future studies on cultivar improvement with this crop.
- iv. develop biochemical and molecular markers for mango and Citrus.

b. Facilities

The current plant tissue culture facilities are unsuitable for this type of research and should be upgraded. The laboratory does not provide enough space for an expanded programme. Moreover, the flow of activities within the tissue culture unit is widely dispersed and needs consolidation. Most importantly, the level of cleanliness has to be greatly improved. Thorough cleansing of walls, ceilings and floors with a solution of sodium hypochlorite (bleach) should be followed by painting of all surfaces with a hard, deck-type enamel that could be rubbed clean easily. Currently, the laboratory would not meet the standards for carrying out studies on transformation according to current US/NIH/APHIS and government of India Department of Biotechnology guidelines.

2. Division of Floriculture and Landscaping

a. Research Program

Two ornamental crop species have been identified as targets for the Indo/USAID subproject, i.e., roses and gladiolus. Considerable effort has already been focused on various aspects of morphogenesis in carnation, with the eventual goal of recovery of useful variant off-types or plants that have been genetically transferred for useful traits. In many respects, carnation has been a useful model, and several interesting regeneration pathways have been described from shoot tip and various of callus. Preliminary studies that will lead to genetic transformation have been accomplished. Future work should focus on description of regeneration pathways from somatic tissues of rose, e.g., somatic embryogenesis from callus derived from immature zygotic embryos and possibly from leaves of in vitro grown plants and organogenesis from callus derived from leaf, petiole and other organs. Highly efficient regeneration protocols that are preferably in liquid phase should be explored.

In anticipation of cultivar improvement schemes, preliminary studies involving isolation and culture of protoplasts should be undertaken. It is also important that different strains of

Agrobacterium tumefaciens should be assembled in order to determine optimum genotype for rose and/or carnation. Collaborative links with researchers currently involved in genetic transformation of ornamental species should be established. Agreements for exchange of engineered vectors and plasmids must be in place well before this activity commences. Reference to the US NIH/APHIS and Government of India Department of Biotechnology guidelines.

Virus diseases of rose can be problematic in many cultivars. Therefore, a scheme should be initiated to ensure that all rose material that emerges from the laboratory, either as an existing cultivar or as an improved cultivar, should be indexed for the known viruses of rose.

Although gladiolus is part of this subcontract, and has been identified as being of importance within the Division of Floriculture and Landscaping, there has been apparently no research on this crop to date. Therefore, protocols for efficient adventitious regeneration from corn tissue, meristems and from inflorescences must be researched. Meristem tip culture, possibly in conjunction with thermotherapy for elimination of viruses should be undertaken. Because viruses are a personal problem in gladiolus, it only makes sense that new cultivars that emerge from this programme should be virus-indexed.

b. Facilities

The existing facilities for plant cell and tissue culture of ornamental crop species urgently need upgrading. There is not enough laboratory space to accommodate an expanded programme of research. This is true for the media preparation area and for the growth room/transfer room. Since relatively sophisticated cell culture procedures and molecular biology approaches are considered to be an integral part of the proposed programme, the laboratories should be appropriate for these types of research, both in terms of size but more importantly with respect to cleanliness and the ability to provide some level of containment. The entire tissue culture unit should be upgraded to at least a P1 level according to US NIH/APHIS guidelines and the Government of India Department of Biotechnology recommendations. Currently, the laboratory should not be involved in any studies involving recombinant DNA, i.e., genetic transformation of plant tissue cultures, due to the absence of basic containment and the need for improving overall laboratory cleanliness and hygiene. In planning a new laboratory for this division, the above guidelines should be followed closely.

.B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

There are many challenges that the group implementing this project will face at this location and these are discussed below.

1. Program Isolation

The IIHR complex is located 30 km outside the city of Bangalore and is not connected to the city by any form of public transportation. This severely limits the work schedule of scientists working at this facility from 9 am to 4:30 pm corresponding to the institute shuttle service. This schedule will severely limit the type of experiments that can be conducted. Some provision needs to be made to facilitate work beyond working hours currently adopted at the institute. Most of the gene transfer experiments need and demand a greater flexibility with time. In addition telephone and data lines are not available to establish good communications with the rest of India. Telephone and data communications need to be improved to provide ready access to other scientific institutions like IISC (Indian Institute of Science at Bangalore), to national data bases, computer networking and FAX. These electronic services will better integrate this location with the rest of the project occurring at other institutions.

Linkage to IISC should be fostered either through joint programs in area of teaching or research. This would provide the opportunity of greater contact between researchers at IIHR and IISC. IISC has strong programs in the area of cell and molecular biology and therefore could be a source closeby for 'good advice' and trouble-shooting.

2. Facilities

Presently the seven researchers cooperating on this proposed project have individual laboratories that are scattered through the main building at IIHR. Optimally these individual units should be collocated as this would provide more convenient access to common equipments and avoid needless duplication of facilities. The collocation would foster a greater interaction among the participants in the project.

The overall conditions of the building facilities indicated that they were in dire need of routine maintenance, i.e., they were dusty and dirty. This will severely hamper the proposed large scale tissue culture and recombinant DNA work. The facilities should be brought up to the P1 level as prescribed by the NIH guidelines on recombinant DNA research. This is essential to meet the basic safety and containment requirements essential for the proposed project.

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)

1. Coconut

a. Somatic Embryogenesis

Despite early and significant research accomplishments, i.e.,

possible organogenesis from floral primordia explants (1978) and somatic embryogenesis directly from young seedling leaf explants (1983: 1985), research accomplishments since then have been insignificant. Certain problem areas are evident in the coconut morphogenic system. Because the plant has only a single shoot or vegetative meristem, standard approaches to developing a regeneration system based on shoot tip culture are wasteful and destructive. Moreover, because there is usually only a single specimen of an elite tree, destructive removal of explants would be catastrophic. Hence, morphogenesis must be obtained from somatic tissue as explants, i.e., floral inflorescences, young leaves and roots. The approach that was developed at CPCRI with some early success involved the use of inflorescences and young leaves from immature plants. Similar studies elsewhere, e.g., Pannetier and Buffard-Morel (1982: 1986) with young leaves and Branton and Blake (1983) with inflorescences, also described the induction of embryogenic cultures of this species. Neither the groups in Europe nor the CPCRI group were able to stimulate the formation and growth of subculturable, highly embryogenic callus or calloid masses. Thus, each inductive event resulted in a finite, quite small number of in vitro regenerants.

No group, including the CPCRI unit, has been able to control coconut somatic embryo development in such a way that apparently normal germination and plant recovery can occur. This problem is probably aggravated by the relatively limited numbers of somatic embryos that can be differentiated from a single explant. It is apparent that coconut somatic embryos have developed precociously in vitro, and like all such somatic embryos, have given rise to plantlets that are not viable.

Coconut zygotic embryos appear to be of the orthodox type. The immature zygotic embryo has a distinct and long period of developmental arrest, during which it fails to enlarge, but remains embedded in the solid endosperm phase. In recent years, it has become apparent that for a large number of species having orthodox-type seeds in which an embryogenic morphogenic system is available, the recovery of normal plants via a normal germination sequence can only be achieved if somatic embryos are stimulated to go into developmental arrest. Usually, this is accomplished by treatment with ABA (abscisic acid) during the early stages of somatic embryogeny. Other treatments that have been successful include treatment with high osmolarities. For every species, there appears to be a critical time during somatic embryogeny when such a pulsed treatment is effective; it is usually during the early to late heart stage period of development. By relating the effects of dormancy inducing treatments to deposition of fatty acids, and by comparing similar levels in zygotic embryos, it may be possible to determine a workable regeneration scheme. [It is noteworthy that coconut somatic embryos are morphologically aberrant, in that they do not possess a haustorium. Such developmental anomalies are often associated with precocious germination of somatic embryos in other

species.] Clearly, the coconut embryogenic system would benefit from clear thinking from the perspective of classical growth and development and from modern concepts that have emerged from the field of seed physiology. Other promising research lines would be to address problems of plant recovery during the acclimatization phase (*ex vitro*). There is good evidence that an environment consisting of 20,000 ppm CO₂ with a high light intensity (80-100 $\mu\text{mol m}^{-2} \text{sec}^{-2}$) will stimulate autotrophy in many plantlets that develops *in vitro*.

b. Embryo Culture

Protocols that have been developed for the extraction of coconut zygotic embryos from newly harvested nuts, field-sterilization, transportation to the laboratory and inoculation into growth medium in order to induce development and germination are being developed. Plantlets have already been obtained, although they have not been transferred to the nursery. This technique will be very important for the international exchange of coconut germplasm between national breeding programmes and repositories.

2. Cacao

a. Somatic Embryogenesis

Very little effort is currently being expended with this crop species due to the decline in area planted during the past few years. It is not anticipated that there will be a demand for planting material of elite material during the immediate future. However, in conjunction with a university thesis project, a procedure for inducing embryogenic callus from zygotic embryo explants and from young leaves from mature tree selections has been developed. Regeneration from leaf explants of this species is a significant discovery, and should be studied in greater detail. It is possible that a refined, efficient regeneration system will be required in the future. It would be wise to anticipate that event by having a reliable clonal procedure available.

b. Haploid Culture

Because of the phenomenon of twin embryo formation in cacao seeds, one of the embryos is haploid. The ability to generate haploid and dehaploid plants is an important goal in tree breeding. The production of homozygosity for important horticultural traits is greatly facilitated by this technique. Since the technique is well described by Dublin, among others, it should be pursued in the context of cultivar development of cacao.

3. Oil Palm

The vegetative propagation of oil palm by somatic

embryogenesis has been developed at a number of commercial micropropagation laboratories in the U.K., U.S.A., Costa Rica and Malaysia. As with coconut, this regeneration pathway is the only possible method for clonal propagation. Embryogenic callus has generally been induced from root explants, although immature leaves and young inflorescences have also been used. Unfortunately, due to the intensive commercial interest in the clonal propagation of oil palm, protocols of regeneration have never been published in any detail. Given the interest of the Indian government in oil palm production expansion, there is surely justification for developing basic and highly efficient regeneration from mature, elite oil palm selections.

The CPRCI tissue culture unit has developed embryogenic cultures of oil palm from seedling explants. Limited success in transplanting in vitro plantlets to soil was achieved several years ago. However, for whatever reason this project has assumed a low priority in recent years. No effort has been made to work with tissues from mature trees. It is important that work with this species should be given a higher priority, particularly the induction of somatic embryogenesis from mature tree selections.

D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)

1. Cashew

a. Shoot Tip Culture

Although there have been a few published reports of the propagation of cashew by the stimulation of axillary bud proliferation in vitro, these studies have utilized very juvenile materials. Therefore, there are few useful guidelines for establishing an effective micropropagation protocol for elite selections of cashews. It would be helpful if experimental cashew selections could be grafted onto seedling rootstocks, and maintained in a protected area as small pruned trees. This would facilitate the control of fungae and bacterial contamination, as the entire plant and soil mixture could be drenched with a fungicide at regular intervals. In addition, the application of phytohormones, particularly the cytokinin benzyladenine (BA), would be made much easier. Such treatment with BA has been useful for conditioning the stock plant (and explant) of many woody species, enabling the explant to be more responsive. It would also be much easier to measure the effect of deep shade or absolute darkness as a conditioning treatment for the explant. Above all, it is important that a range of genotypes should be used for the trial studies, because of the importance of genotype-dependent responses.

In vitro micrografting of clonal meristems onto seedling rootstocks could be tried as a means for rejuvenation of the clone. This has been shown to be effective with avocado, and excised shoot tips of the partially rejuvenated scion have responded as juvenile

material and have proliferated rapidly. Professor Pranom Prutpongse of Kasetsart University (Bangkok, Thailand) is approaching the problem of cashew micropropagation in this manner.

b. Somatic Embryogenesis

There have been at least 2 reports of the induction of embryogenic callus from juvenile (cotyledon) explants of cashew. However, no group, to my knowledge, has explored the possibility of the induction of morphogenesis from somatic tissues of mature tree selections. Mango, which is in the same plant family as cashew (Anacardiaceae) has been regenerated by somatic embryogenesis from nucellar tissue cultures. The procedure is straight forward, and involves the culture of either extracted nucellus or the ovule halves (fertilized ovules from which the zygotic embryo has been removed) onto medium containing low concentrations of 2,4-D. A similar approach should be attempted with cashew, taking into consideration the important variables of genotype effects, season and stage of development of the ovules. If the cashew tissue culture programme is to play a role in cultivar development, such a regeneration system would be essential. In the medium - to - long term of this project, protocols for efficient and large-scale production of somatic embryos should be developed. These protocols could then be used for propagating clonal rootstocks and, more importantly, could be used in a somatic cell genetic programme.

E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY

There are challenges in two general areas that this institution must face in order to develop a strong and rigorous program in the designated crops. One is the relative isolated location of the university and the other is the existence of a department of biotechnology as a unique entity with very little connection with the rest of the more disciplinary oriented programs.

1. Isolated Location

Unfortunately very little can be done about this. The YSPMU is located near Solan well outside the city limits and can be only approached by road. Whereas it is somewhat difficult to see this unit thrive as a hot-bed of international science, its unique location provides the solitude and serenity of a monastery, an optimal environment to concentrate and contemplate. The unique agroclimate conditions provide a unique opportunity to study a wide selection of different plants. Efforts should be made to improve or improvise in the area of communication with other devices that would facilitate the most rapid means to communicate with other locations, units and the rest of the world. Mail would be an important issue for example, because a majority of supplies for

recombinant DNA work are highly perishable, i.e.. restriction and modifying enzymes, radioactive isotopes etc.

2. Program

The department of biotechnology is an independent unit and certainly one of a kind. Most scientists have had problems with the term 'biotechnology' because it is a vague term. We strongly felt a clear lack of communications between biotechnology and the major departments, i.e.. vegetable crops, fruit crops and floriculture. There is great value in having an integrated program where breeders, production physiologists, horticulturists can work together with the biotechnology group. One senses a strong definition of turf at this institution, which in the long run would be counterproductive in terms of this project whose success is dependent on a productive interaction. For example we did not see any interaction with plant pathology in the virus program.

V. TECHNICAL PROGRAM: IMPLEMENTATION OF RESEARCH COMPONENTS

Please refer to the earlier section for a more detailed description of the components of proposed research (A to F) and the individual research objectives. In addition the technical program described below is a result of an evaluation of the individual centres also described earlier.

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

1. Division of Fruits and Horticultural Technology

a. Micropropagation, Regeneration and Field Evaluation of Desirable Genetic Stocks

i. Mango (Fig. 1)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials representing different north Indian cultivars. De novo regeneration via somatic embryogenesis and organogenesis, particularly from these clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

ii. Citrus (Fig. 2)

De novo regeneration via somatic embryogenesis particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

iii. Tomatoes (Fig. 3) and chillies (Fig. 4)

De novo regeneration via organogenesis, particularly from elite cultivars and/or advanced breeding lines.

- b. Advancing Breeding Lines Through Embryo Rescue and Protoplast Fusion Involving Distantly Related Species and the Recovery of Homozygous Dihaploids.

- i. Tomatoes and Chillies (Fig. 3.4)

Embryo rescue for the recovery of advanced genetic materials. Recovery of haploid plants from the culture of anther and ovarian tissues. Protoplast isolation. fusion. culture and regeneration.

- c. Somatic Cell Genetics

- i. Mango (Fig. 1)

Highly efficient morphogenetic suspension and cell cultures for somatic embryogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants.

- e. Technology Transfer and Commercialization

- i. Mango. Citrus. Tomato and Chillies (Fig. 1 to 4)

Identify key industries and grower groups who will be appropriate recipients of the above technologies. Pilot testing of new technologies in collaboration with private and or public agencies.

2. Division of Floriculture and Landscaping

- a. Micropropagation, Regeneration and Field Evaluation of Desirable Genetic Stocks

- i. Gladiolus (Fig. 5)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials and unique cultivars. Meristem culture and micrografting for the production of virus indexed plant materials. Acclimatization, field introduction and evaluation of regenerated plants.

- ii. Rose (Fig. 6)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of specific cultivars. Meristem culture and micrografting for the production of virus indexed plants. De novo regeneration via somatic embryogenesis or organogenesis. Particularly from elite cultivars. Acclimatization, field introduction and evaluation of regenerated plants.

- b. Advancing Breeding Lines Through Embryo Rescue and Protoplast Fusion Involving Distantly Related Species and the Recovery of Homozygous Dihaploids

i. Rose (Fig. 6)

Embryo rescue for the recovery of advanced genetic materials.

c. Somatic Cell Genetics

i. Gladiolus (Fig. 5)

Highly efficient morphogenetic suspension and cell cultures for somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

ii. Rose (Fig. 6)

Development of highly efficient morphogenetic suspension and cell cultures to obtain somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

d. Genetic Engineering

i. Rose (Fig. 6)

Acquisition, amplification, analysis and verification of plasmid vectors and microbial strains to carry out transformation experiments. Agrobacterium-mediated gene transfer and the selection and identification of transformed cultures and regenerants of rose using marker genes and subsequently genes that will modify flower colour. Molecular analysis of regenerants to confirm successful gene transfer and the expression of introduced genes. Field evaluation of transgenic plant materials following the US/NIH/APHIS and government of India Department of Biotechnology guidelines and regulations.

e. Technology Transfer and Commercialization

i. Gladiolus (Fig. 5)

Identify key industries and grower groups who will be appropriate recipients of the above technologies in gladiolus. Pilot testing of new technologies in collaboration with private and or public agencies.

ii. Rose (Fig. 6)

Identify key industries and grower groups who will be appropriate recipients of the above technologies in rose.

Development of research agreements and the protection of intellectual property rights especially for the genes to be used for modification of flower colour. Pilot testing of new technologies in collaboration with private and or public agencies.

B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

1. Program

a. Micropropagation, regeneration and field evaluation of desirable genetic stocks

i. Mango (Fig. 1)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials of mango (Fig. 1). De novo regeneration via somatic embryogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

ii. Banana (Fig. 7)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials of banana. Meristem culture and micrografting for the production of virus indexed plants. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

iii. Citrus (Fig. 2)

Meristem culture and micrografting for the production of virus indexed plants of citrus. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

iv. Tomatoes and Chillies (Fig. 3.4)

De novo regeneration via somatic embryogenesis or organogenesis, particularly from clonal materials of tomatoes and chillies.

v. Gladiolus (Fig. 5)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials of gladiolus. Meristem culture and the production of virus indexed plants of gladiolus. Acclimatization, field introduction and evaluation of regenerated plants.

- b. Advancing breeding lines through embryo rescue and protoplast fusion involving distantly related species and the recovery of homozygous dihaploids

i. Mango (Fig. 1)

Embryo rescue for the recovery of advanced genetic materials from genetic crosses of mango. Protoplast isolation, fusion, culture and regeneration.

ii. Banana (Fig. 7)

Embryo rescue for the recovery of advanced genetic materials in collaboration with breeding program. Protoplast isolation, fusion, culture and regeneration.

iii. Citrus (Fig. 2)

Protoplast isolation, fusion, culture and regeneration.

iv. Tomatoes and chillies (Fig. 3,4)

Embryo rescue for the recovery of advanced genetic materials. Recovery of haploid plants from the culture of anther and ovarian tissues. Protoplast isolation, fusion, culture and regeneration.

c. Somatic cell genetics

i. Mango (Fig. 1)

Development of highly efficient morphogenetic suspension and cell cultures for somatic embryogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

ii. Banana (Fig. 7)

Highly efficient morphogenetic suspension and cell cultures, i.e., somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

iii. Citrus (Fig. 2)

Isolation of efficient morphogenetic suspension and cell cultures capable of somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic

tools for the verification of cultivars and clonal fidelity. somaclonal variants and plant pathogens.

iv. Tomatoes and chillies (Fig. 3.4)

Induction of highly efficient morphogenetic suspension and cell cultures that readily undergo somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity. somaclonal variants and plant pathogens.

v. Gladiolus (Fig. 5)

Highly efficient morphogenetic suspension and cell cultures. i.e.. somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity. somaclonal variants and plant pathogens.

d. Genetic Engineering

i. Mango (Fig. 1)

Acquisition. amplification. analysis and verification of plasmid vectors and microbial strains for mango. Agrobacterium-mediated gene transfer and the selection and identification of transformed cultures and regenerants. Molecular analysis of regenerants to confirm successful gene transfer and the expression of marker genes. Field evaluation of transgenic mango plant materials following the US/NIH/APHIS and government of India Department of Biotechnology guidelines and regulations.

ii. Tomatoes and chillies (Fig. 3,4)

Acquisition. amplification. analysis and verification of plasmid vectors and microbial strains for tomato and chillies. Agrobacterium-mediated gene transfer and the selection and identification of transformed cultures and regenerants. Molecular analysis of regenerants to confirm successful gene transfer and the expression of introduced genes in tissues of tomato and chillies. Field evaluation of transgenic tomato and chillie plant materials following the US/NIH/APHIS and government of India Department of Biotechnology guidelines and regulations.

e. Technology Transfer and Commercialization

i. Mango. Tomatoes and chillies (Fig. 1,3 & 4)

Identify key industries and grower groups who will be appropriate recipients of the above technologies. Development of

research agreements and the protection of intellectual property rights. Pilot testing of new technologies in collaboration with private and or public agencies.

ii. Banana and Citrus and Gladiolus (Fig. 7, 2 & 5)

Identify key industries and grower groups who will be appropriate recipients of the above technologies in mango, tomatoes, and chillies. Pilot testing of new technologies in collaboration with private and or public agencies.

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)

a. Micropropagation, Regeneration and Field Evaluation of Desirable Genetic Stocks

i. Coconut (Fig. 8)

De novo regeneration via somatic embryogenesis particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

ii. Cacao (Fig. 9)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal selections. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

iii. Oil Palm (Fig. 10)

De novo regeneration via somatic embryogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

b. Advancing Breeding Lines Through Embryo Rescue and Protoplast Fusion Involving Distantly Related Species and the Recovery of Homozygous Dihaploids

i. Coconut (Fig. 8)

Embryo rescue for the recovery of advanced genetic materials.

ii. Cacao (Fig. 9)

Recovery of haploid plants from the culture of anther and ovarian tissues.

c. Somatic Cell Genetics

i. Coconut (Fig. 8)

Development of highly efficient morphogenetic suspension and cell for somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers (protein or DNA) and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

ii. Cacao (Fig. 9)

Development of highly efficient morphogenetic suspension and cell cultures for somatic embryogenesis.

iii. Oil Palm (Fig. 10)

Highly efficient morphogenetic suspension and cell cultures to accomplish somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and assorted plant pathogens.

e. Technology Transfer and Commercialization

i. Coconut, Cacao and Oil Palm (Fig. 8, 9 & 10)

Identify key industries and grower groups who will be appropriate recipients of the above technologies. Pilot testing of new technologies in collaboration with private and or public agencies.

D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)

a. Micropropagation, Regeneration and Field Evaluation of Desirable Genetic Stocks

i. Cashew (Fig. 11)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal selections. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

b. Somatic Cell Genetics

i. Cashew (Fig. 11)

Development of highly efficient morphogenetic suspension and

cell for somatic embryogenesis or organogenesis. In vitro and ex vitro screening for spontaneous and induced somaclonal variants. Identification of molecular markers (protein or DNA) and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

c. Technology Transfer and Commercialization

i. Cashew (Fig. 11)

Identify key industries and grower groups who will be appropriate recipients of the above technologies. Pilot testing of new technologies in collaboration with private and or public agencies.

E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY

1. Program

a. Micropropagation, Regeneration and Field Evaluation of Desirable Genetic Stocks

i. Apple (Fig. 12)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal materials. Meristem culture and micrografting for the production of virus indexed apple plants will be a major focus. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of regenerated plants.

ii. Walnut (Fig. 13)

Shoot-tip and nodal culture for rapid propagation and in vitro preservation of clonal selections. De novo regeneration via somatic embryogenesis and organogenesis, particularly from clonal materials. Acclimatization, field introduction and evaluation of in vitro propagated walnut plants.

iii. Tomatoes and Chillies (Fig. 3 & 4)

De novo regeneration via organogenesis, particularly from elite cultivars.

b. advancing breeding lines through embryo rescue and protoplast fusion involving distantly related species and the recovery of homozygous dihaploids

i. Tomatoes and Chillies (Fig. 3 & 4)

Embryo rescue for the recovery of advanced genetic materials.

Recovery of haploid plants from the culture of anther and ovarian tissues. Protoplast isolation. fusion, culture and regeneration.

c. Somatic Cell Genetics

i. Apple (Fig. 12)

Highly efficient morphogenetic cell cultures for organogenesis of various apple cultivars. In vitro and ex vitro screening for spontaneous and induced somaclonal variants arising in regenerated plants. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

ii. Walnut (Fig. 13)

Highly efficient morphogenetic cell cultures for somatic embryogenesis of different walnut cultivars. In vitro and ex vitro screening for spontaneous and induced somaclonal variants arising from germinated somatic embryos. Identification of molecular markers and other diagnostic tools for the verification of cultivars and clonal fidelity, somaclonal variants and plant pathogens.

d. Technology Transfer and Commercialization

i. Apple. Walnut. Tomatoes and Chillies (Fig. 12,13.3 & 4)

Identify key industries and grower groups who will be appropriate recipients of the above technologies. Pilot testing of new technologies in collaboration with private and or public agencies.

VI. TECHNICAL PROGRAM: PERSONNEL AND TRAINING

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

1. Division of Fruits and Horticultural Technology

a. Personnel Needs

It would be impossible for a single scientist to address both mango and Citrus and to meet the stated research goals. Therefore. additional staffing is important.

Research Scientist	1	(for Citrus)
Technical Assistant	1	(for Citrus)

b. Training

Little progress has been made with either mango or Citrus.

Therefore, training in all aspects of the subproject objectives for these crop species is essential. The following scientists are identified:

i. All aspects of Citrus tissue culture

Dr. Fred Gmitter
Citrus Research and Education Center
University of Florida
Lake Alfred, FL USA

ii. Somatic embryogenesis, regeneration

Dr. Richard Litz
Tropical Research and Education Center
University of Florida
Homestead, FL USA

2. Division of Floriculture and Landscaping

a. Personnel

Research Scientist	1	(molecular biology/genetics)
Research Scientist	0.25	(plant pathology)

b. Training

The researchers in this division have a firm background in plant cell and tissue culture. It would be very useful if there were an opportunity for one or more researchers to work in an ornamental horticulture unit that is specializing in biotechnology for cultivar improvement. This would be useful in at least 2 ways. It would enable them to understand the close relationship that must exist between the commercial objectives of a powerful floriculture industry and the scientific objectives of a research in this discipline. It would also be important to learn some of the molecular biology protocols that are currently being used to improve ornamental crops. Ideally, this programme should be multidisciplinary, with significant interaction among virologists, tissue culturist and a geneticist/molecular biologist. Training should be pursued with one or more of the following scientists:

i. Cell biology, senescence

Dr. Randy Woodson
Department of Horticulture
Purdue University
W. Lafayette, IN USA

ii. Chromosome manipulation. flower colour

Dr. Robert Griesbach
 USDA/ARS
 Floriculture and Nursery Crops Lab.
 Beltsville. MD USA

iii. Protoplast fusion. somatic hybridization

Dr. Ken Sink
 Department of Horticulture
 Michigan State University
 E. Lansing. MI USA

B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

1. Personnel Needs

This unit is quite well staffed they need support staff who will work closely with the research scientists. In addition they need to work closely with the plant breeding group.

Research Assistant	4
Lab./Field Assistant	4

2. Training

Training in all aspects of the subproject objectives for these crop species is essential. The following scientists are identified:

a. Protoplast. culture. fusion

Dr. Jude Grosser
 Citrus Research and Education Center
 University of Florida
 Lake Alfred. FL USA

b. Somatic embryogenesis. regeneration

Dr. Richard Litz
 Tropical Research and Education Center
 University of Florida
 Homestead. FL USA

c. Gene transfer. recombinant DNA

Dr. Abhaya M. Dandekar
 Department of Pomology
 University of California
 Davis. CA USA

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)

1. Personnel Needs

In addition to the existing personnel, a plant pathologist should work in conjunction with the tissue culture group to ensure that MLO-infected coconut palm selections are not introduced into a future micropropagation programme. Disease indexing is essential.

2. Training

It is important that liaison should be established with other laboratories that are focusing on palm tissue culture (irrespective of species) and on tropical tree species that are difficult-to-regenerate. Among others, the following researchers should be considered critical for the programme:

a. Embryogenesis, tissue culture of Cacao

Dr. Jules Janick
Dept of Horticulture
Purdue University
West Lafayette
Indiana 47907

b. Somatic embryogenesis, regeneration of plantation crops

Dr. Richard Litz
Tropical Research & Education Center
University of Florida
Homestead, FL, USA

c. Somatic embryogenesis of Coconut

Dr. Jennet Blake
Wve College
University of London
Ashford, Kent, U.K.

D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)

1. Personnel Needs

None in the immediate future.

2. Training

Mr. Thimmapaiah, the scientist in charge of the tissue culture programme has approached the difficult problem of cashew regeneration in a logical manner. Because his academic and

scientific training were not in the field of plant cell, tissue and organ culture, it is likely that he may encounter considerable difficulties ahead. It is important that he should receive further training in this field. Suggested contacts include:

a. Micropropagation and rejuvenation of Cashew

Dr. Pranom Pratpongse
Dept of Horticulture
Kasetsart University
Bangkok, Thailand

b. Somatic embryogenesis, regeneration

Dr. Richard Litz
Tropical Research & Education Center
University of Florida
Homestead, FL, USA

c. Tissue culture, micropropagation

Dr. Philippe Boxus
La Station de Fruits Mar.
Gembloux, Belgium.

E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY

1. Personnel Needs

None at this time.

2. Training

Personnel at this unit will need training in the areas of propagation and regeneration of walnut and apple.

a. Somatic embryogenesis and regeneration of apple

Dr. David J. James
Horticulture Research International
East Malling, UK

b. Propagation of walnut

Dr. Gale H. McGranahan
Dept. of Pomology
University of California
Davis, CA USA

VII. TECHNICAL PROGRAM: EQUIPMENT NEEDS

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

1. Division of Fruits and Horticultural Technology

a. Equipment: imported

CO2 Incubators - BOD type with light provision and programmable temperature adjustment. (2 units)	\$ 4.000
Millipore Ultrafiltration unit with spares.	\$ 3.000
Stereomicroscope with fibre optics and photomicrographic attachment.	\$ 7.000
Electrophoresis equipment with power supply and spare accessories.	\$ 3.000
Inverted microscope with phase contrast, UV Fluorescence.	\$ 9.000
Refrigerated Microcentrifuge.	\$ 7.000
Eppendorf micropipettes.	\$ 2.000

b. Equipment: Indigenous

Air Conditioners	\$ 2.000
Horizontal Autoclave stainless steel	\$ 1.500
Magnetic Stirrer-cum-Heater	\$ 300
Stand by Generator diesel	\$ 4.000
Personal Computer	\$ 3.500
Orbital Shaker	\$ 4.000
Table-top Centrifuge. low speed	\$ 200
Sub Total	\$ 50,500

A much larger plant tissue culture laboratory is required that is self-contained and that meets international standards.

2. Division of Floriculture and Landscaping

a. Equipment

Incubator-shaker	1	\$ 3,000
Laminar Flowhood	1	\$ 3,500
B.O.D. Incubators	2	\$ 4,000
Top Pan balance	1	\$ 150
Analytical balance	1	\$ 2,000
Air conditioners	4	\$ 2,000
Refrigerators	1	\$ 1,000
Lux meter	1	\$ 400
Inverted Microscope photomicrography	1	\$ 8,500
Microwave Oven	1	\$ 500
Electrophoresis units for horizontal gels	1	\$ 1,500
Electrophoresis units for vertical gels	1	\$ 1,500
Clinical centrifuge	1	\$ 1,000
Personal Computer + printer	1	\$ 4,000
Micropipettes + tips	4	\$ 2,000
	Sub Total	\$ 35,550

B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

1. Tissue Culture.

Mettler Series AE Analytical Balance Capacity 200 g.	\$ 3,000
Leica Diastar Research microscope with fluorescence phase contrast and microphotography attachment.	\$ 15,000
Bausch and Lomb Photozoom inverted microscope.	\$ 5,000
Sanyo Microwave oven Automatic and Programmable.	\$ 1,000
Microwave leakage tester.	\$ 300

Convicon tissue culture rooms.TCR 60 11'x9'x6' light intensity 315 uE/m /s	\$ 25.000
Olympus Stereozoom microscope with photomicrography attachment and fibre optics illumination.	\$ 5.000
Digital micropipettes Eppendorf with with disposable tips.	\$ 750
Labline floor model Shaker. (2 items)	\$ 5.000
Millipore milli Q System	\$ 5.000
2. Molecular Biology and Genetic Engineering.	
Packard Tri-carb 2500 TR Liquid Scintillation counter consisting of:- a) Basic System. b) Software Kit. c) Dot Matrix Printer 80 column bidirectional high speed printer with IBM compatible interface. d) PS/2 30-286 computer with 8950000 Disk Media Kit. e) Colour monitor for 220V.50Hz operation.	\$ 35,000
STRATAGENE Electrophoresis System consisting of:- a) VAGE System. b) Possiblot Pressure blotter. c) Stratalinker UV crosslinker. d) Power pack for 220V.50Hz operation.	\$ 1,000
Sorvall RC-5C Superspeed refrigerated centrifuge (220V.50Hz) with the following accessories:-	\$ 20.000

a) Fixed angle rotors SS-34. GSA. GS	
Fotodyne Hand Held 300nm UV DNA lamp.	\$ 500
Lap top computer Portable comp supersport 12 MHZ 20 MB. 3.5".1.44MB floppy drive. 80287 math coprocessor.	\$ 2,000
Personal computer IBM PC Compatible PC/AT 386 OR 486 with color monitor.	\$ 4,000
Computer software (latest versions). a) Wordperfect Ver. 5.1 b) Ventura. c) dBase IV. d) DOS 4.1.and UNIX/XENIX e) High level language compilers like Basic Turboascal.C. f) Sigma Plot Scientific graph system.	\$ 2,000
Beckman Optima XL-70 Preparative Ultracentrifuge for 220V.50Hz phase 30 Amp.. with the following accessories:- a) Type 70.1 Ti rotor c) Type NVT 65 Rotor f) Tool Kit for 70Ti rotor Accessories like Quick-seal tubes, capped tubes etc. Tube cap Vise etc.	\$ 75,000
Pipetman with Tips	\$ 2,000
Labline Imperial III serological baths	\$ 2,000
Rival Ice-O-matic	\$ 1,500
Incubator BOD.Low temperature Labline Ambi-Hi-Co	\$ 1,135

Savant centrifugal drier	\$ 4.000
3. Virology.	
37 CO2 incubator	\$ 3,500
Quixell cell transfer system (for monoclonal antibody)	\$ 3.000
Bioreactors (monoclonal antibody prodn.)	\$ 3.500
Mammalian cell culturing system (Acusyst)	\$ 3,000
Sub Total	\$228.185

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)

1. Facilities and Equipment Requirements

Growth chamber	\$ 3.500
CO2 incubator	\$ 3.500
Microfuge. refrigerated	\$ 5.000
Inverted microscope	\$ 9.000
Roller drum equipment for suspension culture	\$ 3.000
Flask rotary shaker	\$ 3.000
Spectrophotometer	\$ 9.000
Freezing microtome	\$ 3.000
Adjustable micropipettes (Gilson)	\$ 2.000
Millipore vacuum filtration unit	\$ 500
Vacuum pump	\$ 1.500
Air conditioners (2)	\$ 2.000
Hot water bath (for melting media)	\$ 500
Power supply	\$ 1,500
Tank for electrophoresis.	\$ 500
Sub Total	\$ 47.500

D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)

None requested.

E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY

Stereozoom microscope with fibre optics (two)	\$ 14.000
ELISA plate reader	\$ 15.000
Cryocans	\$ 2.000
Electrophoresis equipment with power supply.	\$ 2.000
'Milli Q' equipment to pure water.	\$ 3.000
CO ₂ incubator.	\$ 2,000

UV and phase contrast microscope	\$ 18.000
Sub Total	\$ 56.000
Total Equipment	----- \$417,685 -----

VIII. TECHNICAL PROGRAM: FIVE YEAR BUDGET

The below mentioned bugetary figures represent a total for a five year project.

A. INDIAN AGRICULTURAL RESEARCH INSTITUTE (IARI)

1. Division of Fruits and Horticultural Technology	
a. Total Equipment	\$ 50,500
b. Training	\$ 21,500
c. Consultancies	\$ 21,400
d. Consumable Supplies	\$ 60,000
Sub Total	\$ 153,400
2. Division of Floriculture and Landscaping	
a. Total Equipment	\$ 35,550
b. Training	\$ 32,500
c. Consultancies	\$ 21,400
d. Consumable Supplies	\$ 60,000
Sub Total	\$ 148,950

B INDIAN INSTITUTE OF HORTICULTURAL RESEARCH (IIHR).

a. Total Equipment	\$ 228,185
b. Training	\$ 32,500
c. Consultancies	\$ 21,400
d. Consumable Supplies	\$ 120,000
Sub Total	\$ 402,085

C. CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (CPCRI)		
a.	Total Equipment	\$ 47,500
b.	Training	\$ 21,500
c.	Consultancies	\$ 21,400
d.	Consumable Supplies	\$ 60,000
	Sub Total	\$ 150,400
D. NATIONAL RESEARCH CENTRE FOR CASHEW (NRCC)		
a.	Total Equipment	\$ -- --
b.	Training	\$ 21,500
c.	Consultancies	\$ 21,400
d.	Consumable Supplies	\$ 30,000
	Sub Total	\$ 72,900
E. DR. Y.S.PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY		
a.	Total Equipment	\$ 56,000
b.	Training	\$ 21,500
c.	Consultancies	\$ 21,400
d.	Consumable Supplies	\$ 60,000
	Sub Total	\$ 158,800

	GRAND TOTAL	\$1,086,535

IX. TECHNICAL PROGRAM: MONITORING ARRANGEMENTS

A. MONITORING ARRANGEMENTS

It is appropriate that peer review of the progress of the proposed research programmes should be directed by ICAR, perhaps through the offices of the DDG (H). Progress reports by the principal investigator at each research location would be prepared at 6-month intervals. These reports would fully discuss salient features of the study and any bottlenecks that might have arisen.

The DDG (H) would designate one or more collaborators/consultants abroad, who would be asked to review the project reports critically. These reviews would be returned to the office of the DDG (H), who would advise each group accordingly.

One and a half years after the projects have been initiated and again after 3 years, the designated foreign collaborators/consultants would be invited to review the progress of each research programme. He would report verbally to the research group and by written report to the office of the DDG (H).

It is expected that research that is supported at this level should generate a significant number of scientific publications in internationally recognized journals. In addition, because of patentable nature of some of this work, every measure should be made to protect intellectual rights.

B. INTERFACING COLLABORATORS

Many of the procedures and technologies that would be developed in this research programme could be exploited relatively quickly and affect horticultural production with a short time-lag. However, these accomplishments cannot be simply transferred to a farmer by the scientist. The technologies should be commercialized by private institutions that have a keener sense of the market place. Many research institutions that already exist in India could take this responsibility. These include Indo-American Seed, A.V. Thomas and possibly T.E.R.I. (Tata Energy Research Institute). In technology transfer, the government should retain its right to exercise ownership or licensing of new technologies.

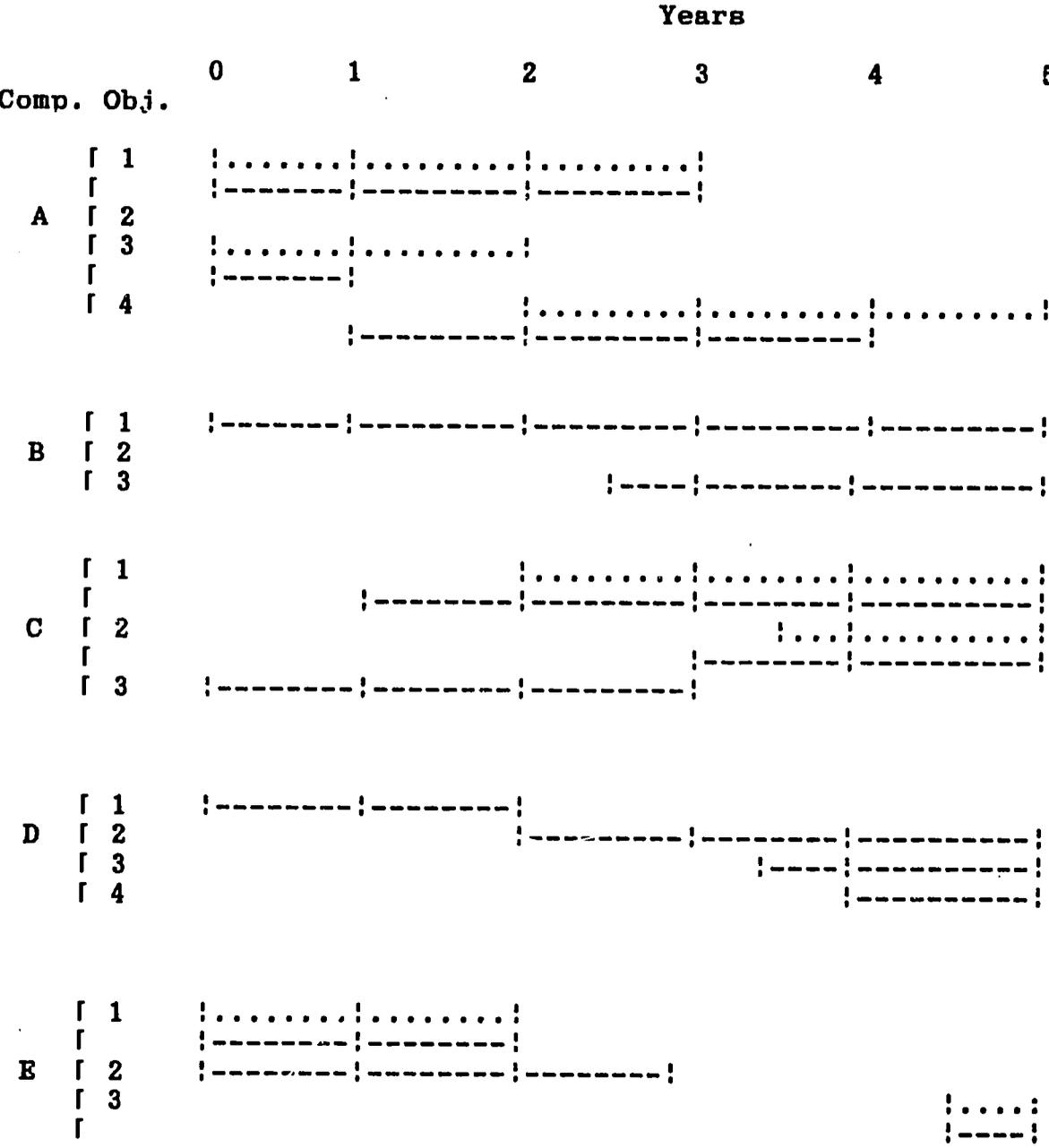
Table 2.

Research Components & Specific Objectives
at Participating Institutions

INSTITUTION	CROP	Research Components																
		A				B			C			D				E		
		1	2	3	4	1	2	3	1	2	3	1	2	3	4	1	2	3
IARI Delhi	Mango	+	-	+	+	-	-	-	+	+	-	-	-	-	-	+	-	+
	Citrus	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+
	Tomato	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+
	Chillies	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+
	Gladiolus	+	+	-	+	-	-	-	+	+	+	-	-	-	-	+	-	+
	Rose	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
IIHR Bangalore	Mango	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
	Banana	+	+	+	+	+	-	+	+	+	+	-	-	-	-	+	-	+
	Citrus	-	+	+	+	-	-	+	+	+	+	-	-	-	-	+	-	+
	Tomato	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
	Chillies	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gladiolus	+	+	-	+	-	-	-	+	+	+	-	-	-	-	+	-	+
CPCRI Kasaragod	Coconut	-	-	+	+	+	-	-	+	+	+	-	-	-	-	+	-	+
	Cacao	+	-	+	+	-	+	-	+	-	-	-	-	-	-	+	-	+
	Oil Palm	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+
NCCR Puttur	Cashew	+	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+
Y.S. PARMAR UNIVERSITY Solan	Apple	+	+	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+
	Walnut	+	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+
	Tomato	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+
	Chillies	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+

Figure 1. Schedule of Research Components and Specific Objectives for Mango at IARI and IIHR.

MANGO



IARI
 IIHR -----

Figure 2. Schedule of Research Components and Specific Objectives for Citrus at IARI and IIHR.

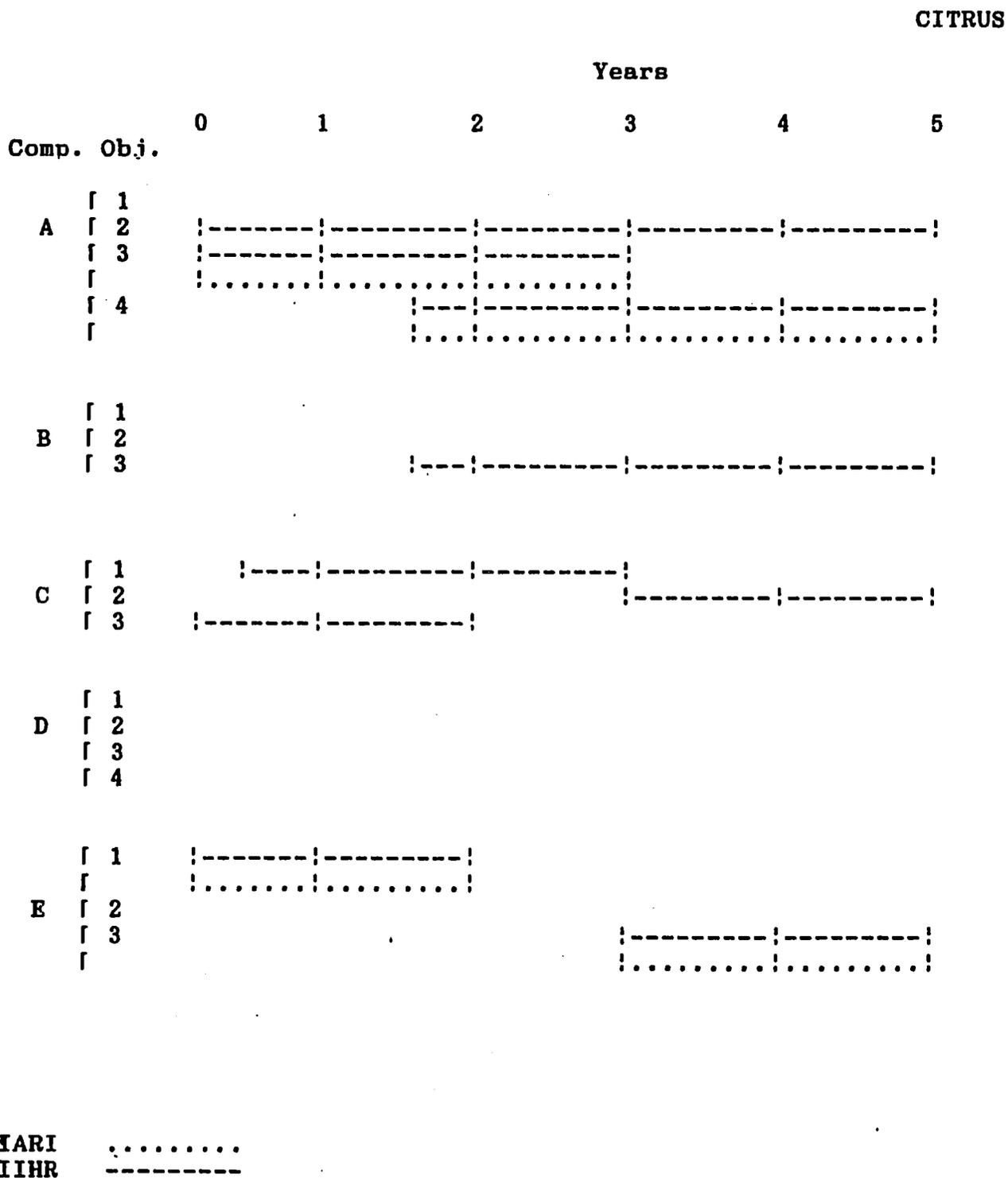


Figure 3. Schedule of Research Components and Specific Objectives for Tomatoes at IARI, IIHR and YSPU.

TOMATOES

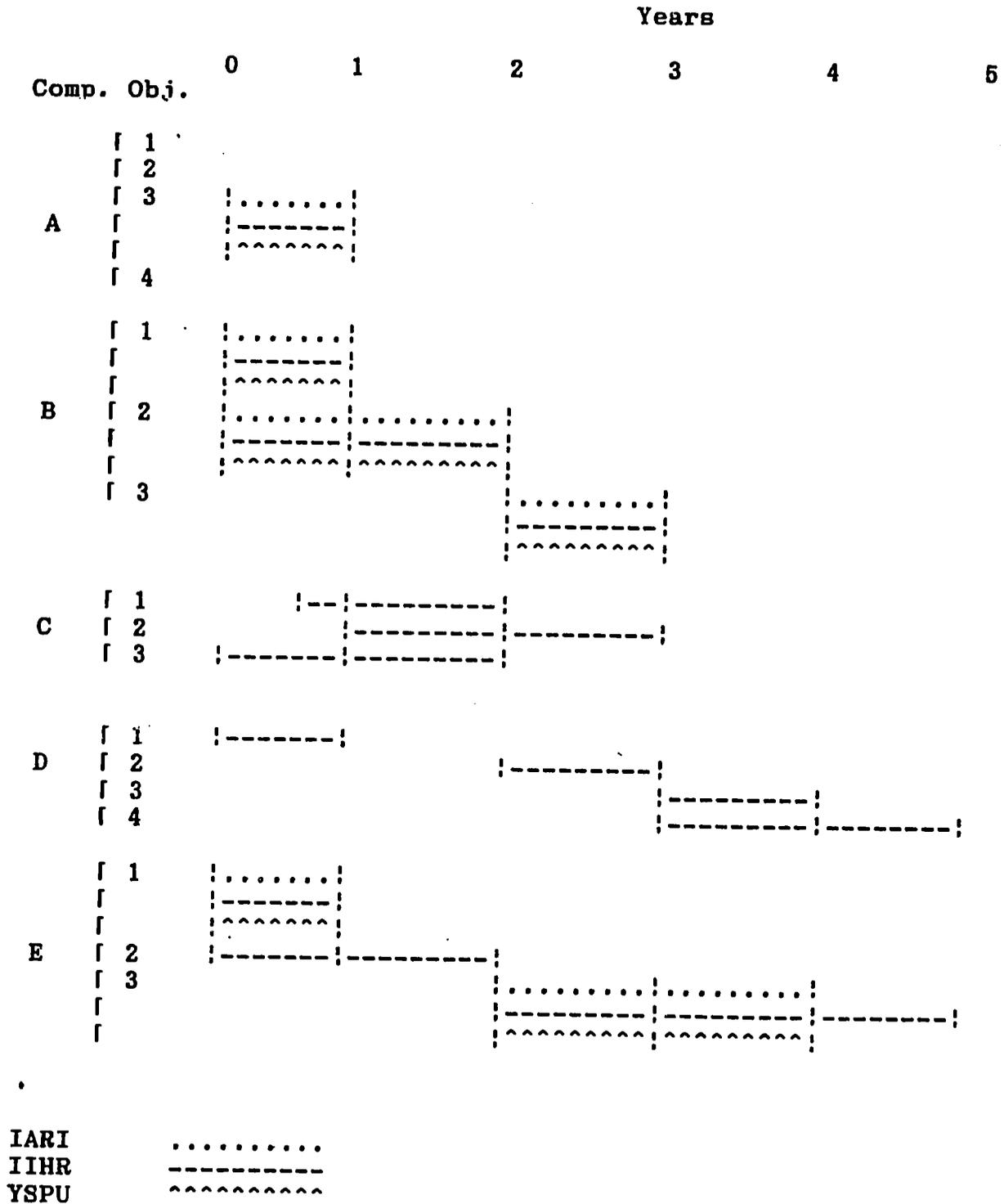


Figure 4. Schedule of Research Components and Specific Objectives for Chillies at IARI, IIHR and YSPU.

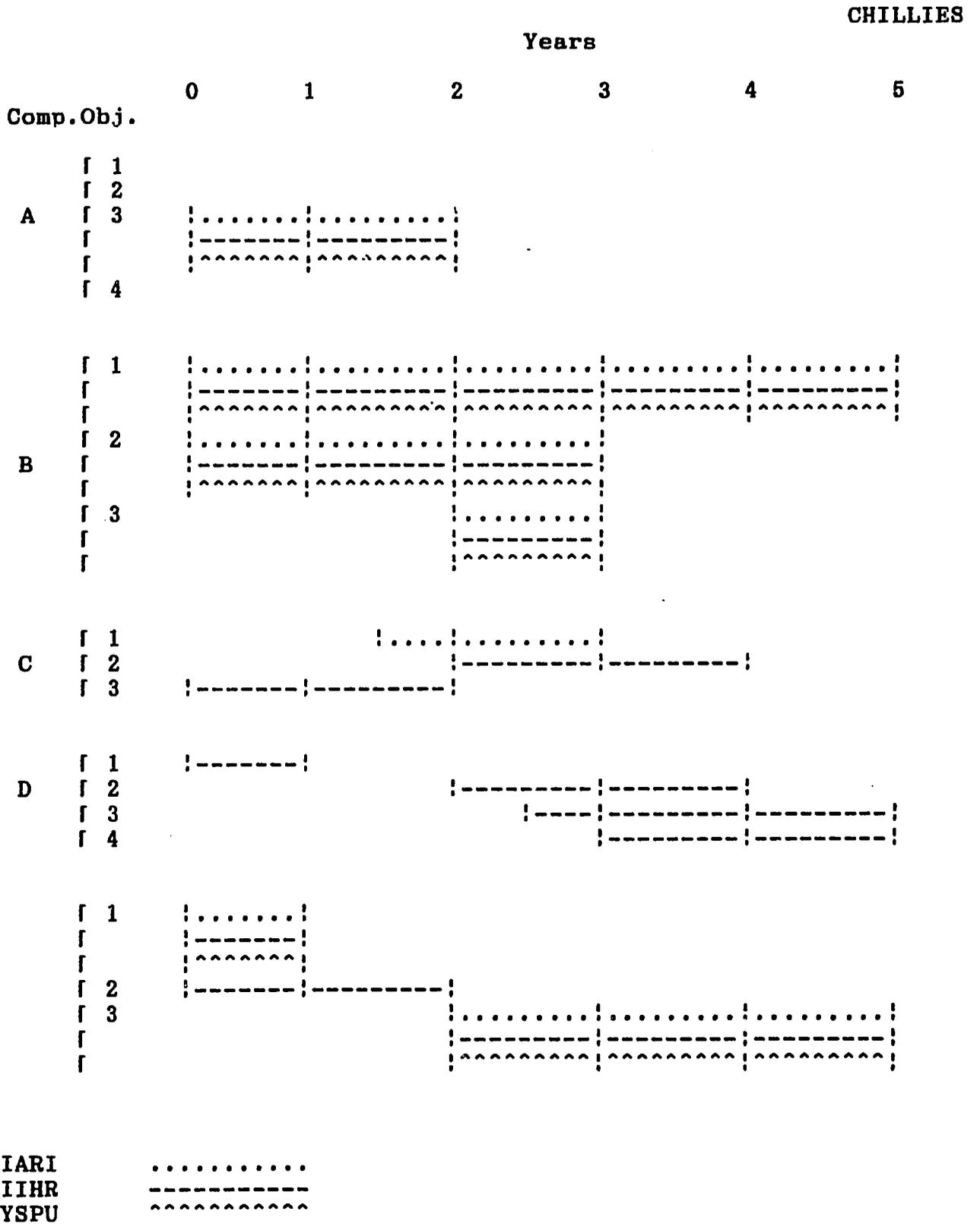


Figure 5. Schedule of Research Components and Specific Objectives for Gladiolus at IARI and IIHR.

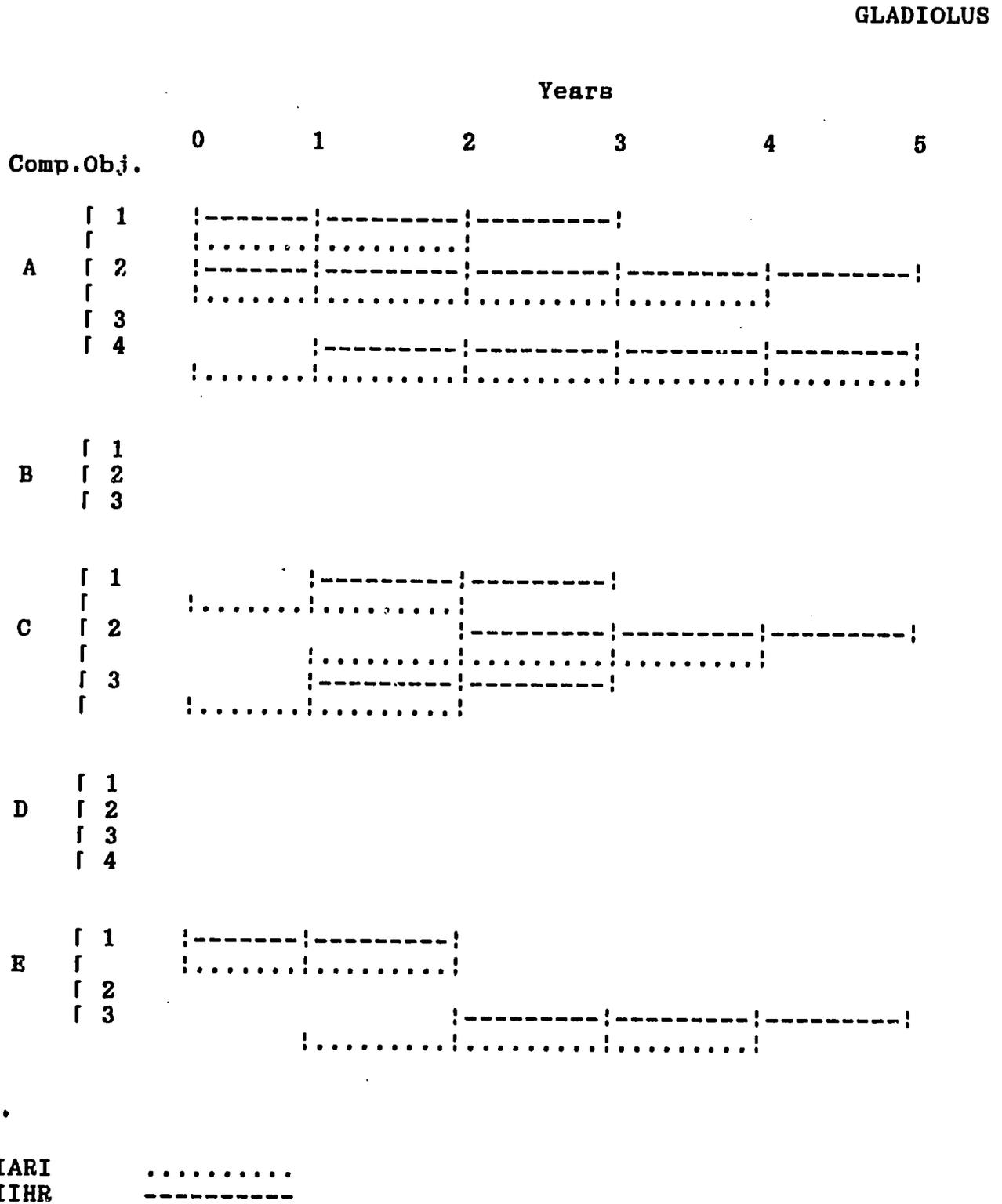
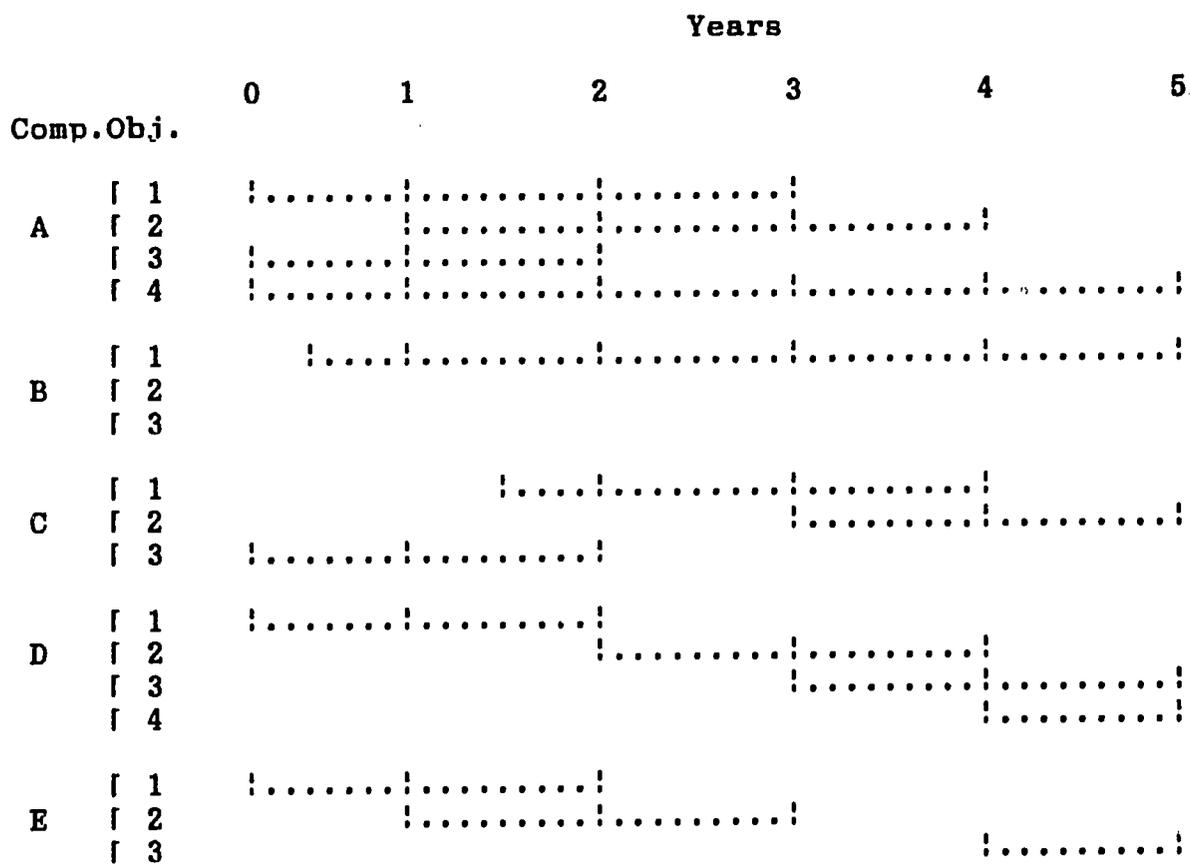


Figure 6. Schedule of Research Components and Specific Objectives for Gladiolus at IARI and IIHR.

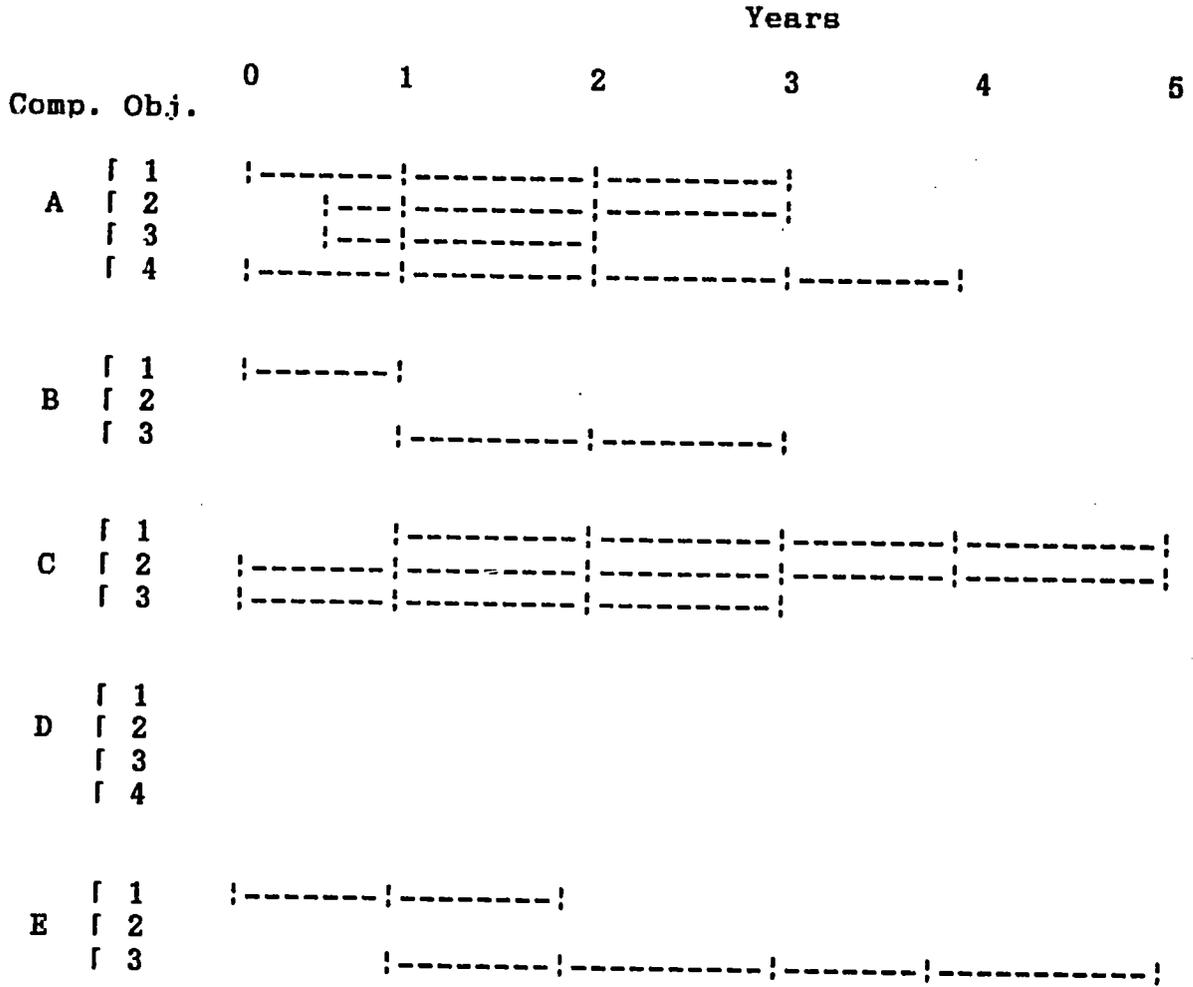
ROSE



JARI

Figure 7. Schedule of Research Components and Specific Objectives for Banana at IIHR.

BANANA



IIHR -----

Figure 8. Schedule of Research Components and Specific Objectives for Coconut at CPCRI.

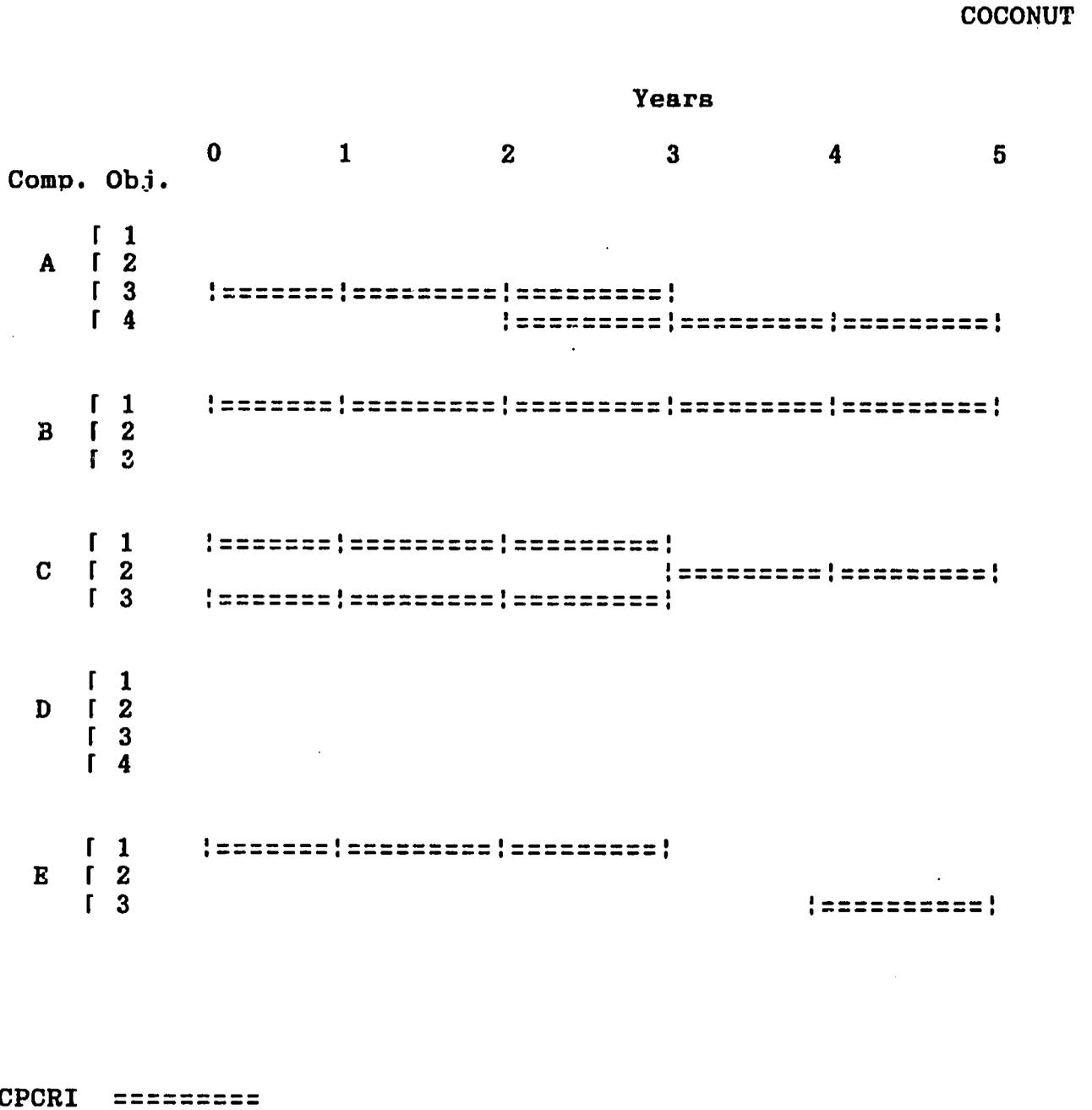


Figure 9. Schedule of Research Components and Specific Objectives for Cacao at CPCRI.

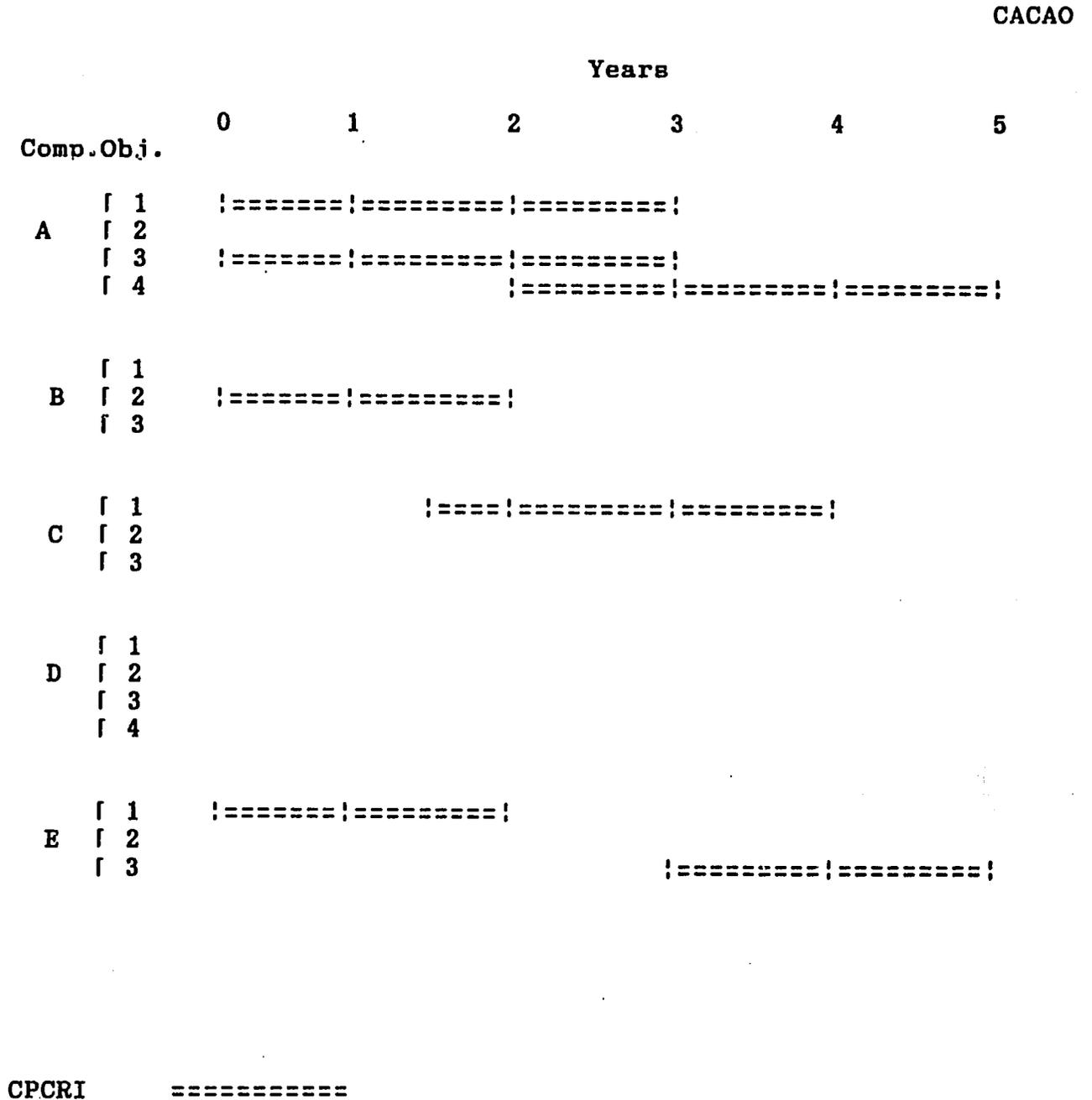


Figure 10. Schedule of Research Components and Specific Objectives for Oil Palm at CPCRI.

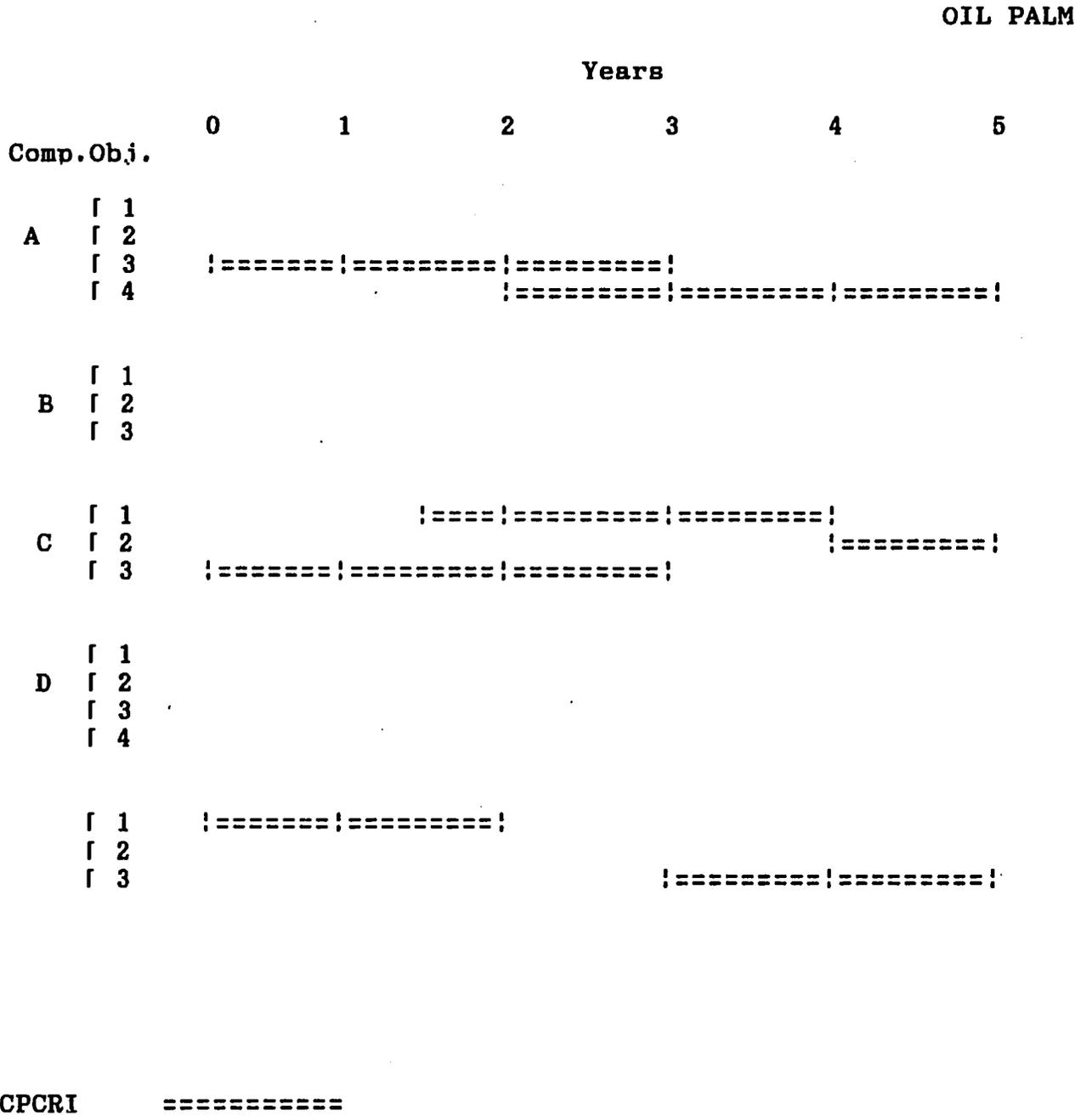


Figure 11. Schedule of Research Components and Specific Objectives for Cashew at NRCC.

CASHEW

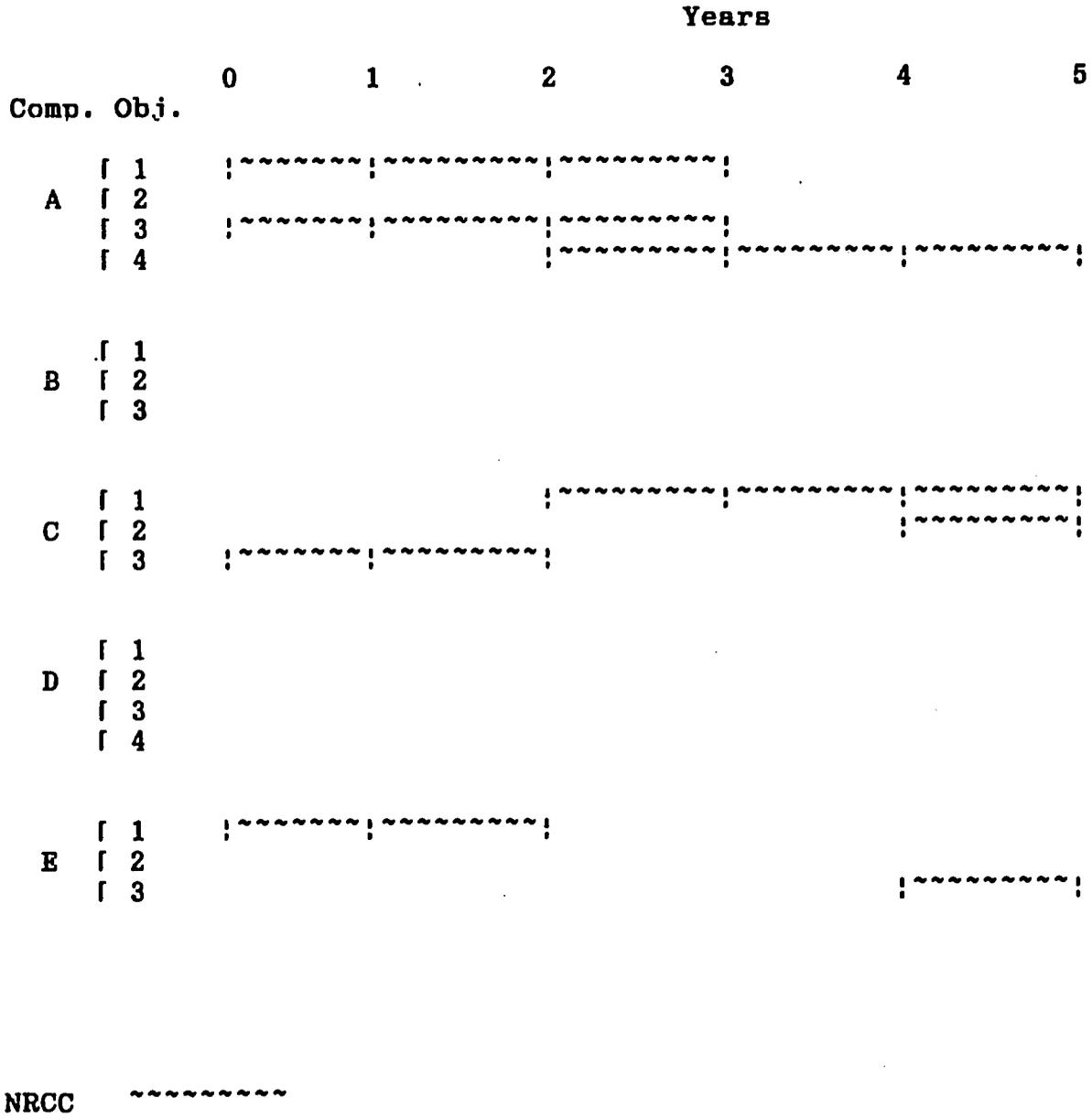


Figure 12. Schedule of Research Components and Specific Objectives for Apple at YSPU.

APPLE

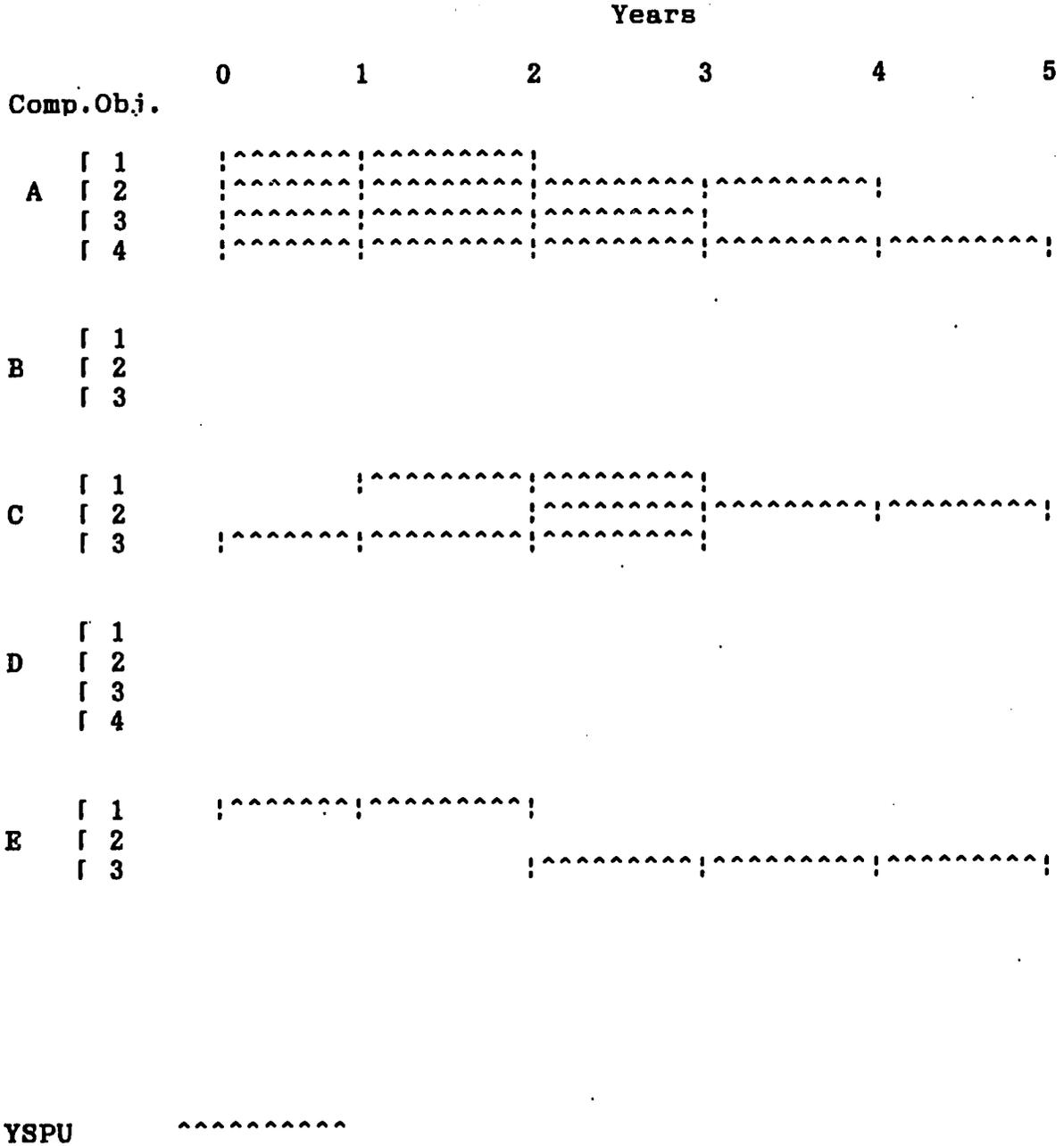
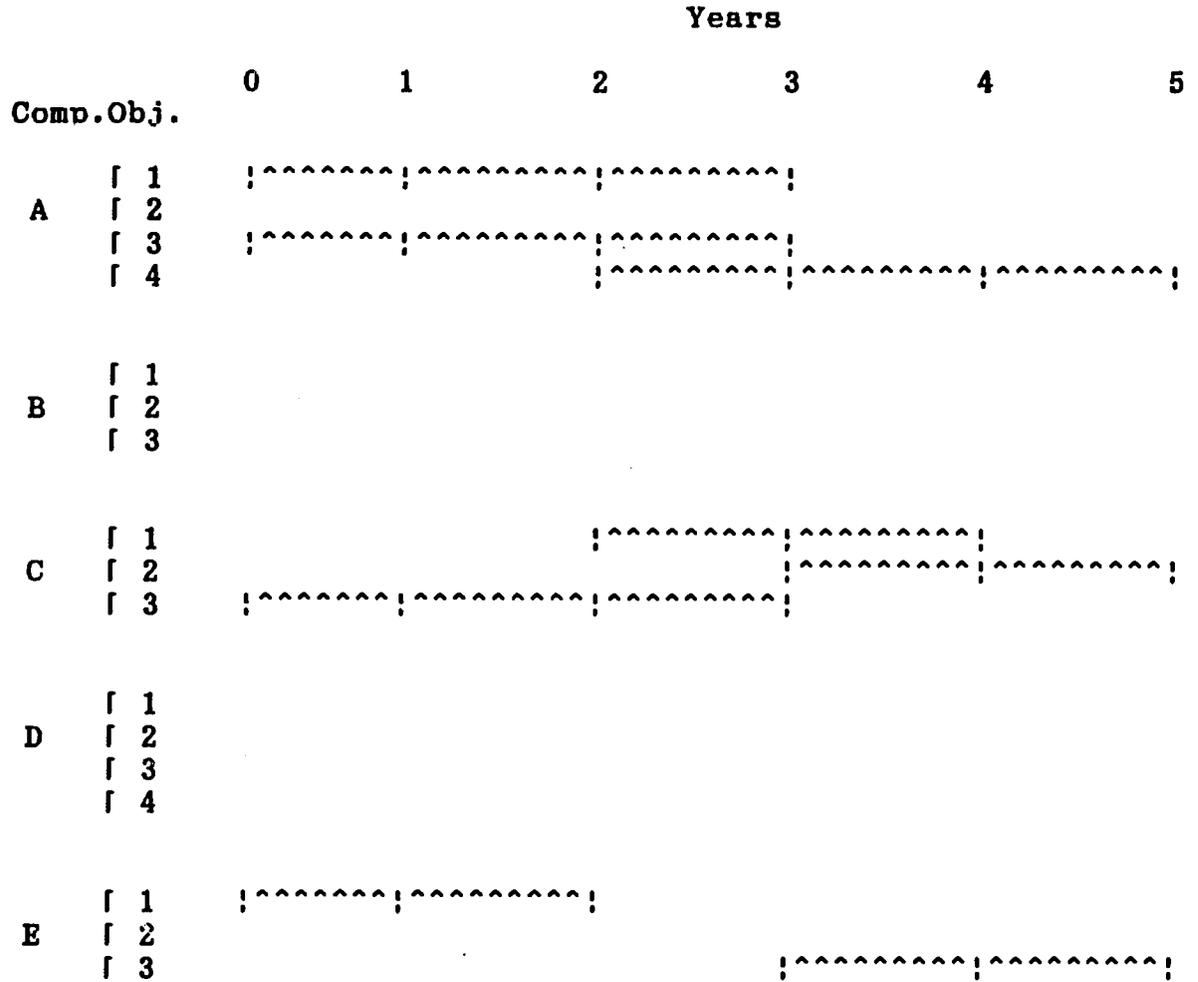


Figure 13. Schedule of Research Components and Specific Objectives for Apple at YSPU.

WALNUT



YSPU ^ ^ ^ ^ ^ ^ ^ ^

TISSUE CULTURE

Scope of Work for the Design Team

1. OBJECTIVES

Specific objective of the project is to up-grade the capability of the Indian research system in terms of research infrastructure, competence of the scientists and development of appropriate research programs in the area of tissue culture/biotechnology for faster multiplication of planting material of elite varieties and genetic improvement of horticultural crops.

2. STATEMENT OF WORK

- i) The team will review the research activities in India on use of tissue culture for micro-propagation and biotechnological approaches involving work in the area of molecular biology and genetic engineering for the improvement of crops in general and of horticultural crops in particular.
- ii) The team will visit the 4 research centers identified for the project, namely, IARI, IIHR, CPCRI and YSPUH & F to review their work, achievements made, research methodology being used, etc. and also assess their capabilities for the enlarged research programs envisaged.
- iii) The team will also evaluate research infrastructure and manpower capability available at each center and suggest appropriate upgradation.
- iv) The team will also identify specific problems in the crops identified for the project which may be amenable to biotechnological approaches for their resolution and suggest a suitable research program.

- v) Based on the above review and interaction with the scientists, the team will develop a document in consultation with Indian counterparts acceptable to ICAR and containing the following:
- a) Summary of the team's review of the research on tissue culture and biotechnology relevant to horticulture crops;
 - b) Identification of specific problems to be solved and the strategy to address the problems in respect of each identified crop;
 - c) Identification of international scientists and institutions which could provide training and other support and expertise in such fields;
 - d) Summarize the on-going research activities, research infrastructure and the scientific manpower resource available at the 4 centers, and suggest specific plan of action for up-grading their standards for improving their output;
 - e) Develop cost estimates of the jobs involved including additional staff, equipment, training and technical assistance required at each center and for the project as a whole;
 - f) Develop an implementation schedule for the project activity;
 - g) Propose organizational including monitoring arrangements required for the project implementation, and also suggest a feasible collaborative tie-up, if required, for each center with other institutions involved in similar or closely related activities in the country.

ITINERARY FOR

DR. RICHARD E. LITZ. PROFESSOR. UNIVERSITY OF FLORIDA. USA
DR. A.M. DANDEKAR. ASSOC.PROFESSOR. UNIVERSITY OF CALIFORNIA. USA

SHORT-TERM CONSULTANTS FOR THE DESIGN TEAM
UNDER INDO/USAID PRE-PROJECT ACTIVITY ON 'TISSUE CULTURE'

DEC. 01 (SUN) : ARRIVE NEW DELHI VIA LH-760 AT 01:20 HRS.
STAY : TAJ PALACE HOTEL, NEW DELHI

DEC. 02 (MON) : AM. BRIEFING AT WINROCK/USAID/ICAR
PM. DELHI/BANGALORE IC-403 1750/2030

DEC. 03 (TUE) : VISIT TO IIHR. BANGALORE
DEC. 04 (WED) : VISIT TO IIHR. BANGALORE
STAY : TAJ RESIDENCY HOTEL, BANGALORE

DEC. 05 (THUR) : BANGALORE/MANGALORE IC-559 0720/0800
VISIT TO CPCRI. KASARAGOD

DEC. 06 (FRI) : VISIT TO CPCRI. KASARAGOD
STAY : CPCRI GUEST HOUSE, KASARAGOD

DEC. 07 (SAT) : MANGALORE/BOMBAY IC-160 1155/1310
BOMBAY/DELHI IC-187 1500/1655
STAY : TAJ PALACE HOTEL, NEW DELHI

DEC. 08 (SUN) : DELHI/SOLAN BY ROAD (RENTAL CAR)
DEC. 09 (MON) VISIT TO DR. Y.S.PARMAR UNIVERSITY OF
HORTICULTURE AND FORESTRY. SOLAN (H.P)
DEC. 10 (TUE) : AM. VISIT DR.Y.S.PARMAR UNIVERSITY
STAY : UNIVERSITY GUEST HOUSE. SOLAN
PM. SOLAN/DELHI BY ROAD 1300/2000
DEC. 11 (WED) : VISIT TO I.A.R.I.. PUSA. NEW DELHI
DEC. 12 (THUR) : AM. VISIT TO NBPGR. IARI CAMPUS. PUSA
PM. DISCUSSION AT ICAR WITH INDIAN
COUNTERPARTS
DEC. 13 (FRI) : PROJECT DOCUMENT PREPARATION
DEC. 14 (SAT) : PROJECT DOCUMENT PREPARATION
DEC. 15 (SUN) : IN DELHI OR TRIP TO AGRA
DEC. 16 (MON) : PROJECT DOCUMENT PREPARATION
DEC. 17 (TUE) : MEETING AT ICAR WITH DRS. K.L. CHADHA, DDG
(HORT) AND G.L. KAUL, ADG (HORT)
DEC. 18 (WED) : PROJECT DOCUMENT FINALIZATION
DEC. 19 (THUR) : PROJECT DOCUMENT FINALIZATION
DEC. 20 (FRI) : DE-BRIEFING MEETING AT WINROCK AND USAID
DEC. 21 (SAT) : DEPARTURE FROM NEW DELHI

If allocation of the total budget figure is not made by USAID, although a portion of the budget is made available, we recommend that distribution be made along institutional lines rather than along commodity lines. This would enable one or more institution to develop a center of excellence that will ensure completion of certain components and objectives of this project.

Therefore, to facilitate future decision that will be made in this regard, we give below our priority list of the participating institutions :

1. IIHR
2. CPCRI
3. IARI (Horticulture Division)
4. Y.S. Parmar University, Solan
5. NRCC
6. IARI (Horticulture Division)