BIOTECHNOLOGY - PLANT PROPAGATION BY TISSUE CULTURE

1. Introduction

Plant tissue culture or micropropagation technology has made invaluable contribution to agriculture by enabling the production of disease free, quality planting material of commercial plants and fruit trees, throughout the year. It is a technique for in-vitro growth of plantlets from any part of the plant in a suitable nutrient medium under controlled aseptic conditions.

Commercial tissue culture industry is in existence globally since the last 25 years. However, in India, plant tissue culture industry started about 15 years back and is revolutionizing the commercial agriculture sector by enabling mass propagation of quality planting material.

The success of agriculture development hinges on selection of desired types of plants and their multiplication. Traditionally, agriculture crops are multiplied by means of seeds (sexual propagation) or organs other than seeds (asexual or vegetative propagation). These organs are usually stems, roots or modified underground structures. Though multiplication by seeds is the cheapest method, it suffers form certain disadvantages. Plants raised from seeds may not repeat good performance of mother plants.

Many horticultural plants take a long time to produce seeds/fruits and many of them do not produce viable seeds or desired quality of seeds. Plants propagated vegetatively do not suffer from these disadvantages. However, vegetative propagation is rather a slow, time and space consuming process. Besides, it is usually infected with latent diseases. Some plants are also not amenable to vegetative method of propagation, for example, coconut, papaya, oil palm, clove etc.

Therefore, scientists started a quest for an alternative method of plant propagation, which could overcome the disadvantages of both the methods described above. After many trials and errors in the sixties, plant propagation by tissue culture method was found commercially successful in the case of orchids. Since then, it has become indispensable in propagation of many valuable crops like banana, sugarcane, papaya etc. Micropropagated plants have been well accepted by farmers all over the country because of its uniform productivity, free from disease, vigorous growth and high yield. Higher yield is contributed by increase in the yield per plant as well as larger number of plants, which can be cultivated per unit area.

2. Major advantages of Tissue-Culture:

The main advantage of tissue culture technology lies in the production of high quality and uniform planting material that can be multiplied on a year-round basis under disease-free conditions anywhere irrespective of the season and weather. However, the

technology is capital, labour and energy intensive. Although, labour is cheap in many developing countries, the resources of trained personnel and equipment are often not readily available. In addition, energy, particularly electricity, and clean water are costly. The energy requirements for tissue culture technology depend on day temperature, day-length and relative humidity, and they have to be controlled during the process of propagation. Individual plant species also differ in their growth requirements. The commercial advantages of tissue culture technology over its conventional counterpart are summarized below:

- Tissue culture could be a useful way for circumventing or eliminating disease, which can accrue in stock plants.
- Tissue Culture Plants (TCPs) may have increased branching and flowering, greater vigour and higher yield, mainly due to the possibility of elimination of diseases.
- The method may succeed to propagate plants where seeds or vegetative propagation is not possible or difficult or undesirable. As the capital investment on mother plants is reduced to almost zero, it may be easier to adapt to changing conditions. Additionally, a better programming of the production is possible, because of the greater plant uniformity and the availability in the mass at any time.
- Enables storage and maintenance of stock plants/germplasm.

3. Commercial prospects

In India there are approximately fifty established commercial tissue culture units. Their production capacity ranges between 1 million to 5 million and above plants per annum with an aggregate production capacity of 200 million plantlets per year. Most of these tissue culture units are located in Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. These companies have been concentrating on exploiting local as well as the international markets.

Propagation by tissue culture offers good commercial prospect in ornamental plants, vegetables and also fruit plants, where value of the products is high.

In recent years, the demand for TCPs of elite plant varieties has grown tremendously in domestic market. Still the demand is greater for horticultural and forest species. The Working Group on Horticulture and Plantation Crops for the Eleventh Five Year Plan has projected the total requirement of planting material of fruits, coconut, cashew, black pepper, spices, arecanut etc. as 2000 million by the year 2012. It may not be possible to meet this requirement by conventional nurseries. It would, therefore, be desirable to encourage commercial tissue culture labs to supplement the production of planting material. The technologies, which have been perfected for large-scale propagation, are as follows:

Fruits	Banana, grapes, cashew, pineapple, strawberry, sapota, watermelon, apple and citrus.
Cash crops	Sugarcane, potato and tapioca.
Spices	Turmeric, ginger, vanilla, large cardamom, small cardamom, vanilla and clove.
Medicinal plants	*Aloe vera*, geranium, stevia, patchouli, rosemary, gloriosa, tulsi
Ornamentals	Gerbera, carnation, anthurium, lily, syngonium, cymbidium, limonium, dracena, philodendron, rose-miniature, caladium, gentiana and cactus.
Trees	Teak, white teak, bamboo, eucalyptus, populus, pine and red sanders.

4. Tissue culture technology

Tissue culture technology is based on the theory of totipotency i.e. the ability of a single cell to develop into whole plant. The major components of the technology include choice of explant (excised part of plant), growing of explant on a defined medium in glass vessel (in vitro), elimination and or prevention of diseases, providing appropriate cultural environment and transfer of plantlets from glass vessel to natural environment (hardening). All these constitute protocol for tissue culture. It varies from species to species and variety to variety within the same species. However, it can be standardized through trial and error and ultimately it should be repeatable and reliable.

4.1 Stages of Tissue Culture

Propagation by tissue culture is divided into five stages. A general account of these stages is outlined below.

4.1.1 Choice of explant

Explants could be shoot tips (meristem), nodal buds, sections from internodes, leaves, roots, centres of bulbs, corms or rhizomes or other organs. The choice depends on the species to be multiplied and the method of shoot multiplication to be followed. Actively growing (shoot tips), juvenile (seedlings) or rejuvenated tissues (suckers) are preferred.

The commercial tissue culture laboratories commonly use tips of apical or lateral shoots, which contain meristems. Meristems are made up of actively dividing cells in an organized manner. They are about 0.1 mm in diameter and 0.25 - 0.30 mm. in length. However, explants should be chosen from typical, healthy, disease free, well-tested mother plants cultivated under conditions, which

reduce contamination and promote growth of tissues to be cultured. If necessary explants may be subjected to virus testing and elimination. The selection of mother plants is very important for commercial success of tissue culture propagation.

4.1.2. Establishment of Germfree (aseptic/sterile) culture

Excised part of plant is surface sterilized and transferred to sterile nutrient medium contained in glass vessel. On an average, about 25 cc nutrient media may be added per glass vessel. The cultures are maintained in growth rooms. If there is no infection and tissue isolated from mother plants survive in the artifical environment, initiation of new growth will take place after a week or so. Thus, germ-free culture is established.

4.1.3. Production of shoots/propagules

Once growth is initiated by induction of meristematic centres, buds develop into shoots by multiplication of cells. There are three types of multiplication systems for production of shoots.

i) Multiplication by axillary shoots

In this case shoots are produced from excised shoot tips or nodes. Commonly hormones (cytokinins) are used to induce multiple branching wherein, the rate of multiplication is low. Still it is preferred, because axillary shoots are likely to be genetically stable and the chances of production of types unlike mothers are less.

ii) Multiplication by adventitious shoots

Explants such as sections of leaves, internodes or roots can produce directly adventitious shoots or other organs. This system has higher multiplication rate, but lesser genetic stability than axillary system.

iii) Multiplication by somatic embryos (embryoids)

Embryos are usually formed by the union of male and female reproductive cells (zygotic embryo), which ultimately can develop into a young plant. Embryo - like structures can also be produced from somatic cells. Somatic embryos are independent bipolar structures

and are not attached to the tissues of origin. They can also develop to form young plants like zygotic embryos. Somatic embryos may be produced directly from explants such as sections of leaves, internodes or roots on solid culture medium.

The formation of young - plants mentioned under (i) and (ii) above, or formation of somatic embryos, mentioned in the preceding para, directly on excised plant parts occurs only in certain species.

The most common form of regeneration of plants occurs indirectly from callus. Callus is a mass of undifferentiated dividing cells often formed in tissues cultured in-vitro. Callus may give rise either to adventitious shoots, which develop into plantlets, or somatic embryos, which develop into seedlings. Callus is formed even naturally in response to wound.

Selecting proper tissue and culture medium can induce the formation of callus. This system has the highest multiplication rate and produce complete tiny plants. One gram of explants can produce one lakh somatic embryos. Dormancy can be induced in them or they can be transformed into synthetic seeds. However, callus is genetically unstable or plants arising from it may be unlike mother plants. Such plants are known as off-types. They occur more frequently in callus culture and adventitious shoot culture as compared to axillary shoot culture. Off-types are undesirable in commercial propagation. Regeneration of shoots or intact plants by any one of the multiplication systems described above is influenced by many factors, such as composition of medium (specially concentration of growth regulators), type of tissue, genotype, ploidy level, etc.

Normally, multiplication cycle i.e., the period from incubation of plant parts on medium to formation of shoots varies from 3 to 6 weeks. However, the process is recycled many times by sub-culturing in order to obtain required multiplication rates. After completion of a cycle, shoots are cut separately and transferred to fresh medium. Cutting is done manually by using dissecting tools in laminar flow cabinets, where the air is clean to prevent any contamination. Once the shoots are placed on fresh medium, they are transferred back to the growth rooms. Thus, it may be possible to multiply the shoots 3 to 10 times per cycle of 3 to 6 weeks duration.

4.1.4 Preparation of micro-cuttings for establishment in the natural environment.

Young axillary or adventitious shoots are finally separated from clusters (micro cutting) for initiation and development of roots. After separation, they are transferred individually to a medium containing rooting hormone (auxin) and continued to be maintained in the growth rooms until the roots are formed. It may also be possible to transfer the micro cuttings directly to soil or compost in humid

green house for root formation. Somatic embryos may directly develop into seedlings.

4.1.5 Establishment in the natural environment

The most critical stage in propagation by tissue culture is the establishment of the plantlets in the soil. The steps involved are as under

- washing of media from plantlets,

- transfer of plantlets to compost/soil in high humid green house,

- gradual decrease in humidity from 100% to ambient levels over 3-4 weeks,

- and gradual increase in light intensity.

Plantlets during their growth in laboratory do not photo synthesize and their control of water balance is very weak. They use sugar contained in medium as source of energy. They exist like bacteria (heterotrophs). They need to be converted to more plant like existence (autotrophs) i.e., they should be in a position to utilize carbon-di-oxide from the air and solar energy for their food requirement. This acclimatization on the harsh real environment, outside artifical laboratory milieu takes place gradually.

4.2. Culture environment

Environment conditions in the growth room, which influence cell multiplication, are light, day length and temperature. In tissue culture, light is required for synthesis of green pigment (chlorophyll) and development of organs. The range of light intensities appropriate for culture room varies from 1000 to 5000 lux. Requirement of day length would be in the range of 16-18 hours. Temperature requirement varies from 20 – 30°C depending on species of plants. Tropical plants may require higher temperature than temperate plants.

Prevention of contamination

Prevention of contamination in tissue culture is extremely important for commercial success of the unit. The entire production can go waste if the culture is contaminated. Sugar rich culture medium, excised plant tissue and culture environment are all conducive to the

growth of pathogens. Therefore, it is essential that all operations be conducted in sterile or aseptic conditions. Various stages involved in prevention of contamination are outlined below: - Mother plants should be grown under conditions which do not promote diseases.

Explants should be free from disease. Meristem, used as an explant, is usually free from disease. Surface sterilization of explants in solutions of sodium or calcium hypochlorite is necessary. Heat or treatment with certain chemicals may eradicate latent viruses. All equipments and culture media are sterilised by autoclaving at 15-lb/sq. inch pressure at 121°C for 15 – 20 minutes. Double distilled water should be used for washing explant and preparation of culture medium. UV lamps assist in sterilisation of laminar flow cabinets, hatches and instruments.

Air handling units are employed for growth rooms and culture transfer rooms in order to avoid cross contamination between different areas of operation inside the clean area. The sterile condition is obtained in laminar airflow cabinets as they are provided with special type of international standard HEPA filters. These filters remove all the dust particles of above 0.3 micron present in the air.

5. Objective of Tissue-Culture Project

The primary objective of tissue culture projects could be propagation of large quantity of good quality planting material from elite mother plants within a short period of time and space.

6. Requirements of Tissue-Culture Project

In line with the technology and objective of tissue cultural propagation, various facilities may also be required for such projects which are indicated below:

6.1 Land:

It is required to set up laboratory, mother plant unit, green house and office. Space may also be required for installing tube well / dug well and parking of vehicles.

6.2 Source of technology:

It would be evident from the general outline of the technology, that propagation by tissue culture is much more sophisticated than other types of plant propagation. A tie-up with reputed laboratories, Indian or foreign, could be helpful. However, if well-qualified and

experienced staff are recruited, it may be possible to set up such units without any tie-up.

6.3 Mother Plants:

Mother Plants would serve as source of tissues (explant). Their performance should be tested before use as source of explant. In case of tie-up with well-established laboratories, explants from tested mother plants could be available free of cost. Otherwise, collection, maintenance and testing of superior mother plants would be necessary.

6.4 Laboratory:

A tissue-culture laboratory generally comprises of media preparation room, media store room, inoculation room, growth room, culture transfer room, sterilization area, washing area, etc. The floor plan should be designed to promote maximum efficiency. The design should facilitate maintenance of optimum temperature, humidity, illumination and ventilation.Correct design of a laboratory will not only reduce contamination, but also achieve high efficiency in work performance. Properly planned and designed laboratories can reduce both the operational and energy costs. A tissue culture laboratory must be designed to accommodate the equipment and its use in the various stages of micropropagation in the most efficient manner.

6.5 Equipments:

Propagation by tissue culture needs a good number of laboratory equipments. The various equipments and their functions are outlined below:

i) Autoclave:

Sterilisation of all glass apparatus and culture media can be accomplished by means of steam generated in the autoclave.

ii) Analytical/Top Pan balances:

For accurate measurement of various constituents of culture media, these balances would be required. Top pan balance is used for measuring larger quantities, while analytical balance is used for measuring smaller quantities.

iii) pH meter:

It is used for measuring and adjusting hydrogen ion concentration of the culture media or solution. Hydrogen ion concentration needs to be maintained accurately for achieving optimum growth of plants.

iv) Laminar Air-flow cabinets:

In these cabinets shoots developed on explants are separated from clusters and transferred to fresh medium under sterile condition. Inoculation can also be done here.

v) Distillation sets:

Water to be used for preparation of culture media should be free from all impurities and salts. This can be accomplished by double distillation of water.

vi) Computer System:

Computerisation of laboratory in the following aspects would be helpful. Production planning time scheduling of sub-culturing, quality control of plantlets, growth room status, material requirement, market planning etc.

vii) Air Conditioners with Stabilizers:

Maintenance of desired temperature in growth room, inoculation room/culture transfer room would be possible by air-conditioning these areas.

viii) Microscopes:

a) Stereo Microscope: This would enable dissecting out small size meristem from shoot tips by removing the protective covers of leaf primordia.

b) Compound microscope: This enables detection of bacteria and fungi in culture and plant tissues.

ix) Bottle Washing Unit:

Since a large number of bottles or vessels in which plants will be grown are required to be washed repeatedly before use, an automatic bottle-washing unit would be helpful.

x) Media Cooking Unit:

Culture media, which contains all the essential nutrients, sugar and agar needs to be cooked before use. A media-cooking unit for a large scale commercial unit is, therefore, desirable.

xi) Growth room racks:

These hold the culture bottles in trays. They are mobile over a set of rails in order to maximize utilization of space.

xii) Trays:

Supporting structure for culture bottles/vessels.

xiii) Hatches:

Pass through boxes used as gateway between clean area and semi-clean area for exchanging materials.

xiv) Tube lights:

Fluorescent tube lights are mounted on the bottom of the shelves so that culture bottles containing explants/growing tissues receive requisite intensity of lights.

xv) Dissecting Kits:

These are necessary for separation of shoots and preparation of micro cuttings.

Apart from the above, equipments such as refrigerator, rotary shakers, a stand by Genset, fire extinguisher, oven, air filters and furniture would be necessary. The office should have facilities such as fax machines, telephone and computers etc.

6.6 Culture Media:

The medium in which plant tissue grows is made up of various salts (containing all the major and micro elements essential for growth of plants), vitamins, sugars (usually sucrose) and growth regulators at appropriate concentration. Of the various constituents of the medium, the concentration of growth regulators is critical. The plant growth is virtually controlled by the ratio between two groups of growth regulators. Cytokinin group favours shoot growth, whereas auxin group favours root growth. The ratio varies between species and even between varieties within a species.

Therefore, proper choice of media and containers can reduce the cost of micropropagation. The replacement of expensive imported vessels, alternatives to gelling agents, use of household sucrose, and some medium components can reduce cost of production. Preparation of media in bulk and storage as deep frozen stocks also reduces labour costs.

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6.7 Green House

In tissue-culture propagation, green house may be required to raise and maintain mother plants so that growth of organs suitable for tissue culture is maximum particularly in case of ornamentals and to harden the plantlets gradually in natural environment. Green house will enable the control over light intensity and humidity, which is necessary for hardening of plants. However, in case of export-oriented projects, it may be possible to export the ex-agar plantlets directly from the laboratory without subjecting them to hardening.

6.8 Electricity:

As it would be evident from the preceding paragraphs, no tissue-culture laboratory can operate without electricity. Electricity is essential to provide required intensity of light to the growing tissues and shoots while they are in laboratory, to operate various

equipments and facilities, which include air conditioners.

6.9 Water:

The various purposes for which water is essential are indicated below: Water for growing mother plants, hardening of plantlets, washing, canteen, toilets, etc. Distilled water is required for preparation of culture media and reagents.

6.10 Raw materials

Raw materials required for tissue-culture project, apart from explant, are various constituents of culture media. These have already been discussed under paragraphs 4, 6.3 and 6.5.

6.11 Skilled Manpower:

Tissue culture is a highly skilled operation. It would, therefore, be essential that laboratory and green house workers are well qualified and experienced in the technology. Their training in well-established commercial laboratories would be helpful.

7. Location

Tissue culture project for export may be located near to airports. The site should be well connected with roads. Assured source of water and supply of electricity to the site are essential.

8. Unit Size.

The size of tissue-culture project could be expressed in terms of the capacity of production of tissue-cultured plants (TCPs). The projects so far set up in our county with assistance from financial institutions have production capacities, which vary from 1 million to 20 million TCPs per year. The size envisaged in the present model is 5.0 million TCPs per year. It has been estimated that, to produce 5.0 million TCPs, a laboratory of 6500 sq. ft. would be required. A green house facility of 15000 sq. ft. for maintenance of mother plants and hardening tissue-cultured plants would be helpful.

9. Project Cost

The estimated project cost for production of 5.0 million plants is Rs.181.23 lakh. Details of the project cost are given in Annexures I, I(a), I(b), I(c), I(d), I(e) and I(f). The techno economic parameters assumed in the model scheme are given in Annexure II. The details of manpower requirement and recurring expenditure are given in Annexures III & IV. The actual laboratory production of plantlets will be commencing from second year, hence the margin money for working capital has been capitalized during second year. However, it may be noted that above estimates are subject to actual drawings and rate analysis by competent architects for all civil structures and quotations from accredited dealers for all equipments, furniture, etc.

10. Projected Benefit.

The total production of TCPs is calculated at 80% capacity utilization from 5th year onwards (i.e., 4000000 TCPs). Out of which 80% is saleable at net average price of Rs.6.0 per TCP both at domestic as well as foreign markets.

The construction of civil work, setting up of laboratory equipments and creating other facilities will take 6-9 months time and standardization of the tissue-culture protocol and trial run of production will take 6 months. The actual production will start from 2nd year onwards, therefore no income has been projected in the 1st year. The estimated production of TCPs and the income generation are given in Annexure-V.The depreciation on plant, machinery and other items required is shown in Annexure VI.

11. Market Development

Presently, export-oriented units in tissue culture enter into buy back arrangements with foreign collaborators. Under these arrangements high cost equipments are imported and high fees are paid on know-how even though these are locally available. The buy-back is available only for a limited period of 2-3 years. In the present model it has been assumed that the beneficiaries will develop their overseas markets by visits, publicity, distribution of free samples, etc. Since all materials, equipments and know-how are locally available, it might be possible to produce high quality TCPs at a comparatively low cost.

12. Financial analysis

Financial analysis (Annexure VII) based on Discounted Cash Flow Technique indicates that the project is financially viable, as would be evident from the following data:

NPV at 15% DF - Rs. 150.51 lakh

BCR = 1.27

IRR = 35%

Average DSCR = 1.87

13. Financial assistance

The tissue-culture export-oriented projects are eligible for refinance support by NABARD. Banks may provide loan for the activity provided the scheme is technically feasible and financially viable.

14. Terms of Financial Assistance

14.1 Nature of Beneficiaries

The beneficiaries under the project could be qualified professional entrepreneurs/sole proprietary concerns/partnership firms / public and private limited companies/co-operatives.

14.2 Margin Money

The margin money / down payment prescribed is 20-25%. The cost of land owned or purchased by the borrower upto 10 % of the project cost can be reckoned towards margin money.

14.3 Bank Loan

Bank loan of 75-80 % of the total cost of development shall be available from the financing institution. Bank loan considered in the model is 75%.

14.4 Rate of interest

Banks are free to decide the rate of interest within the overall RBI guidelines issued from time to time. However, the ultimate lending rate has been considered as 12% for working out the bankability of the model project.

14.5 Security

Banks are guided by RBI guidelines issued from time to time in this regard.

15. Repayment

The cash flow statement is furnished in Annexure VIII. Based on the surplus generated, the repayment of principal and interest will start from 2nd year onwards. The entire loan with interest may be repayable over a period of seven years as indicated in Annexure IX.

DISCLAIMER

"The techno-economic parameters including cost, economics, repayment schedule and other terms & conditions are only indicative. While formulating the projects, the entrepreneurs/banks should revise their projects according to the specific situation prevailing in project areas. The rates of interest charged to ultimate borrowers etc., wherever mentioned, are as applicable at the time of preparation of the model schemes and as such, the latest position in this regard can be ascertained from the circulars/guidelines issued by RBI/NABARD from time to time. Further the financial viability and bankability of the scheme may have to be reworked taking in to account the prevailing tax structures and other levies wherever applicable".

Annexure-I

ESTIMATED COST OF INVESTMENT

No.	Particulars	Ref. No.	Cost (Rs. Lakhs)
A)	**Fixed Cost**		
I	Land development	Annexure I (a)	3.95
ii	Tissue culture laboratory including green house and green house equipments	Annexure I (b) & I (c)	45.00
iii	Laboratory equipments	Annexure I (d)	79.64
iv	Furniture, fixtures and office equipments	Annexure I (e)	15.18
v	Water supply system	Annexure I (f)	0.94
vi	Training		2.25
vii	Consultancy / know-how fees		10
	Subtotal		**156.96**
B)	**Preliminary & Pre-Operative Expenses**		**12.21**
C)	**Margin Money for Working capital (25%)**		**7.35**
D)	**Contingency**		**4.71**
	Total		**181.23**
	Project cost		**181.23**
	Bank Loan		**135.92**
	Margin		**45.31**

Note : Margin Money for working capital to be provided in the second year

Annexure - 1(a)

LAND DEVELOPMENT

S.No	Particulars	Area	Rate (Rs.)	Amount in Rs
1	Land development	3 acre	20000	60000
2	Compound wall	Lumpsum		250000
3	Land scaping	5000 sq.ft	7	35000
4	Miscellaneous			50000
	Total			**395000**

Annexure - 1(b)

Civil Structures- Specification of laboratory

	Particulars	Floor Area (sq.ft)	
A.	**Clean Area**		
1	Media Store and Production Control	200	
2	Post Autoclave Area	200	
3	Culture Transfer Room	500	
4	Growth Rooms		
	(i)	750	
	(ii)	750	
	(iii)	750	

5	Change Area	300	**3450**
B.	**Semi-Clean Area**		
6	Legwash	100	
7	Laboratory / Media prepration /Autoclave	500	
8	Wash Area		
	(i) Bottle	250	
	(ii) Plant	250	
9	Store (consumables)	300	**1400**
C.	**Service Area**		
10	Office Lobby, corridor	550	
11	Scientist Room	400	
12	Computer Room	200	
13	Genset Room	150	
14	Canteen	200	
15	Toilet	150	**1650**
	Total		**6500**
	Covered Area (approx.)		**6500 Sq.ft.**

Annexure - 1(c)

CIVIL STRUCTURES - Cost

S.No	Particulars	Area (Sq.ft)	Rate (Rs/sq ft)	Amount (Rs.)
1	Laboratory	6500	400	2600000
2	Auxilary structure	500	400	200000
3	**Polyhouse**(Construction of polytunnel, irrigation system, cost of cooling pad, fans and other electricity work)	15000	80	1200000
4	**Shadehouse**(Including drip-irrigation etc)	20000	25	500000
	Total			**4500000**

Annexure - 1(d)
LABORATORY EQUIPMENTS

Sl.No.	Particulars	No.	Rate per unit	Total (in Rupees)
1	Autoclave	2	475000	950000
2	Balances	4	50000	200000
3	pH meter	5	10000	50000
4	Laminar airflow	10	90000	900000
5	Distillation unit	2	150000	300000
6	Computer System	5	50000	250000

7	Air-conditioners :			
	a) 1.0 tonnes	4	15000	60000
	b) 1.5 tonnes	12	20000	240000
8	Microscopes	4	30000	120000
9	Bottle washing unit	1	200000	200000
10	Media cooking unit	1	200000	200000
11	Growth room racks (20 racks/room)	60	12000	720000
12	Trays (6 trays/shelve)	2880	120	345600
13	Trolleys	15	2000	30000
14	Diesel Genset (62.5 KVA)	1	265000	265000
15	Dissecting Kits and Inoculation instruments	12	15000	180000
16	Refrigerator	2	20000	40000
17	Air handling unit	2	200000	400000
18	Oven	2	25000	50000
19	Rotary Shaker	2	130000	260000
20	Bottles	200,000	5	1000000
21	Lab clothes	30	750	22500
22	Washing machine	1	18000	18000
23	Incinerator	1	25000	25000
24	Fire fighting equipment	5	20000	100000
25	Stabilizers	10	5000	50000
26	Laboratory glass wares			200000

27	Cryopreservation unit	2	250000	500000
28	Tubelights for growth rooms	1920	150	288000
	Total			**7964100**
	Maintenance of plant & Machinery			6741600
				4500000
				11241600

FURNITURE, FIXURES AND OFFICE EQUIPMENTS

Annexure - 1(e)

S.No	Items	Cost(in Rupees)
1	Tables, Chairs and other furnitures	150000
2	Electrical fittings	750000
3	Tube light for offices,lobby etc., (25)	4000
4	Computer Systems (2 units)	100000
5	Fans	7500
6	Fax Machine	30000
7	Telephone	10000
8	Intercom	16000
9	Pick up Van	440000
10	Miscellaneous	10000
	Total	**1517500**

Annexure - 1(f)

WATER SUPPLY SYSTEM

S.No	Items	Cost (in Rupees)
1	Shallow Tube Well	24000
2	Overhead Tank (1000 litres)	30000
3	Pumpset (3 HP)	30000
4	Pump House	10000
	Total	**94000**

Annexure-II

Techno-economic parameters - Commercial Tissue Culture Unit

S.No	Parameters	Particulars
A	**Technical Parameters**	
1	Installed Capacity (Million plantlets)	5
2	Capacity Utilization (%)	
	a. Second Year	40
	b. Third Year	70
	c. Fourth Year	75
	d. Fifth Year onwards	80
3	Consumables (Media/bottle) in ml	25
4	Number of bottles innoculated in one litre Media	40
5	Potting media (Media/slot) in ml	2

6	Breakage of glasswares (%)	10
7	Repairs & Maintenance (%)	10
8	Packages	
	Exagar plantlets/Box -for Export	100
	Number of Box required -for Export	------
B	**Economic Parameters**	
1	Laboratory and Auxilary Structures (Rs/sqft)	400
2	Cost of Polyhouse including Construction of polytunnel, Irrigation system, cost of cooling pad, fans and other electricity work (Rs/sqft)	80
3	Cost of construction of Shadehouse including drip-irrigation(Rs./sqft)	25
4	Cost of Power (Rs. /unit)	4
5	Consumables (Rs/ litre Media)	60
6	Potting media (Rs/plantlet)	0.2
7	Packaging	
	a. Domestic Market (Rs/pack)	5
	b. Export Market (Rs/pack)	10
8	Air freight (Rs/pack containing 100 plantlets)	100
9	Saleable quantity of production (%)	80
10	Sale	
	a. Domestic Market (Share of sale in %)	50
	b. Export Market (Share of Sale in %)	50
11	Sale Price of plantlets	
	a. Domestic Market (Rs./plantlet)	5

	b. Foreign Market (Rs./plantlet)	7
12	Depreciation	
	a. Civil Structures(%)	10
	b. Plant & Machinery(%)	15
	c. Other items(%)	25
13	Insurance (%)	1
14	Tax (%) cess	30
15	Repayment period (including 01 year grace period)	07 years
16	Interest rate (%)	12

Annexure III

MANPOWER REQUIREMENT (@ 100 %)

S.No	Salary	Numbers	Monthly Salary	Salary/year(in Rupees)
1	General Manager (Scientist-in-charge)	1	40000.00	480000.00
2	Managerial (Production -1, Marketing - 1)	3	15000.00	540000.00
3	Supervisors	3	12000.00	432000.00
4	Skilled	16	8000.00	1536000.00
5	Unskilled	12	4000.00	576000.00
	Total salary per annum	**35**		**3564000.00**

Annexure IV

RECURRING EXPENDITURE (in Rupees)

S.No.	Item	Y E A R S				
		1	2	3	4	5 onwards
1	Manpower					
	a) Salaries	756000	1452000	1452000	1452000	1452000
	b) Wages	0	1056000	1824000	1920000	2016000
2	Laboratory consumables	0	345600	604800	648000	691200
3	Greenhouse potting media	0	160000	280000	300000	400000
4	Mother plant	0	50000	50000	50000	50000
5	Power	0	1354618	2370581	2539908	2709235
6	Fuel	0	80000	100000	100000	100000
7	Packaging	0	10000	17500	18750	20000
8	Air freight	0	1000000	1750000	1875000	2000000
9	Administrative					
	a. Printing & Stationery	10000	20000	20000	26667	31667
	b. Postal, Telephone	5000	50000	50000	80000	102500
	c. Travel	100000	100000	200000	200000	200000
	d. Books and periodicals	50000	30000	30000	30000	30000
10	Market Development					

	a. Foreign visits for marketing contract	200000	200000	200000	200000	200000
	b. Publicity abroad	100000	100000	100000	100000	100000
	c. Distribution of free samples		50000	50000	50000	50000
11	Repairs and Maintenance(including replacement of polythylene)	0	112416	112416	112416	112416
12	Insurance	0	140756	120276	103056	88506
13	Breakage of Glasswares and bottles(10% p.a.)	0	120000	120000	120000	120000
		1221000	**6431390**	**9451573**	**9925797**	**10473524**

Annexure V

ESTIMATED INCOME

Year	**Capacity Utilization (%)**	**Total Production of plantlets**	**Sale of plantlets @ 80% of production**	**Gross Income**
		(No.)	**(No.)**	**(Rs. lakhs)**
1	0	0.00	0.00	0.00
2	40	2000000	1600000	96.00
3	70	3500000	2800000	168.00
4	75	3750000	3000000	180.00
5th year onwards	80	4000000	3200000	192.00

Annexure- VI

DEPRECIATION (Rs in lakh)

		YEARS									
S.No.	**Beginning of the year**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	Civil work	45.00	45.00	40.50	36.45	32.80	29.52	26.57	23.91	21.52	19.37
2	Plant & machinery	79.64	79.64	67.69	57.54	48.91	41.57	35.33	30.03	25.53	21.70
3	Other items	16.12	16.12	12.09	9.07	6.80	5.10	3.83	2.87	2.15	1.61
4	**Depreciation**										
5	Civil work	0.00	4.50	4.05	3.65	3.28	2.95	2.66	2.39	2.15	1.94
6	Plant & machinery	0.00	11.95	10.15	8.63	7.34	6.24	5.30	4.50	3.83	3.26
7	Other items	0.00	4.03	3.02	2.27	1.70	1.27	0.96	0.72	0.54	0.40
8	**Total Depreciation**	**0.00**	**20.48**	**17.22**	**14.55**	**12.32**	**10.46**	**8.92**	**7.61**	**6.52**	**5.60**
9	**End of year**										
10	Civil work	45.00	40.50	36.45	32.80	29.52	26.57	23.91	21.52	19.37	17.43
11	Plant & machine	79.64	67.69	57.54	48.91	41.57	35.33	30.03	25.53	21.70	18.44
12	Other items	16.12	12.09	9.07	6.80	5.10	3.83	2.87	2.15	1.61	1.21

Annexure - VII

FINANCIAL ANALYSIS (Rupees in Lakh)

S.No	**Items**										
		1	2	3	4	5	6	7	8	9	10
1	Fixed Cost	173.87	7.35	-							
2	Recurring Cost	-	64.31	94.52	99.26	104.74	104.74	104.74	104.74	104.74	104.74
3	Total Cost	173.87	71.67	94.52	99.26	104.74	104.74	104.74	104.74	104.74	104.74
4	Benefit	0.00	96.00	168.00	180.00	192.00	192.00	192.00	192.00	192.00	192.00

5	Net Benefit	-173.87	24.33	73.48	80.74	87.26	87.26	87.26	87.26	87.26	87.26
6	Disc. rate	15%									
7	Disc. Factor	0.870	0.756	0.658	0.572	0.497	0.432	0.376	0.327	0.284	0.247
8	Disc. Cost	151.20	54.19	62.15	56.75	52.07	45.28	39.37	34.24	29.77	25.89
9	Disc. Benefit	0.00	72.59	110.46	102.92	95.46	83.01	72.18	62.77	54.58	47.46
10	PW Costs	550.91									
11	PW Benefits	701.42									
12	NPV	150.51									
13	BCR	1.27									
14	IRR	35%									

Annexure - VIII

CASH FLOW STATEMENT (in Rs lakh)

S.No	Particulars	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
1	**Installed Capacity**										
	Capacity utilization (in %)	0%	40%	70%	75%	80%	80%	80%	80%	80%	80%
	Capacity utilized (in lakh Nos)	0.00	20	35	37.50	40.00	40.00	40.00	40.00	40.00	40.00
2	**Income**										
	Production of TCPs(in lakh Nos)	0.00	16	29.75	33.75	38	38	38	38	38	38
	Sale of plantlets(in Rs lakh)	0.00	114.00	199.50	213.75	228.00	228.00	228.00	228.00	228.00	228.00
	Total Income	**0.00**	**114.00**	**199.50**	**213.75**	**228.00**	**228.00**	**228.00**	**228.00**	**228.00**	**228.00**
3	**Expenditure**										
	Salaries and wages	7.56	25.08	32.76	33.72	34.68	34.68	34.68	34.68	34.68	34.68
	Laboratory consumables	0.00	3.46	6.05	6.48	6.91	4.00	4.00	4.00	4.00	4.00
	Greenhouse rooting media	0.00	1.60	2.80	3.00	4.00	1.00	1.00	1.00	1.00	1.00
	Mother plant	0.00	0.50	0.50	0.50	0.50	0.20	0.20	0.20	0.20	0.20
	Power @ Rs.4.0/Unit	0.00	13.55	23.71	27.09	27.09	27.09	27.09	27.09	27.09	27.09
	Fuel	0.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Packaging	0.00	0.10	0.18	0.19	0.20	5.73	5.73	5.73	5.73	5.73

	Air freight	0.00	10.00	17.50	18.75	20.00	11.20	11.20	11.20	11.20	11.20
	Administrative	1.65	2.00	3.00	3.37	3.64	3.64	3.64	3.64	3.64	3.64
	Market Development	3.00	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
	Repairs and Maintenance(including replacement of polythylene)	0.00	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
	Insurance	0.00	1.41	1.20	1.03	0.89	2.29	2.29	2.29	2.29	2.29
	Breakage of Glasswares and bottles(10% p.a.)	0.00	1.20	1.20	1.20	1.20	0.70	0.70	0.70	0.70	0.70
	Contingency (includes replacement of roofing material, LDPE for greenhouse)	0.00	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
4	Interest on Working Capital	0.00	2.65	2.64	2.77	2.93	2.93	2.93	2.93	2.93	2.93
5	**Total Expenditure**	**12.21**	**65.44**	**95.64**	**102.08**	**105.86**	**97.28**	**97.28**	**97.28**	**97.28**	**97.28**
6	**Gross Surplus(PBDIT)**	-12.21	48.56	103.86	111.67	122.14	130.72	130.72	130.72	130.72	130.72
7	Depreciation	0.00	20.48	17.22	14.55	12.32	10.46	8.92	7.61	6.52	5.60
8	Interest on Term loan	0.00	17.70	16.55	14.59	10.09	4.66	0.00	0.00	0.00	0.00
9	**Profit after Depreciation Interest (PBT)**	**-12.21**	**10.38**	**70.09**	**82.53**	**99.74**	**115.60**	**121.80**	**123.11**	**124.20**	**125.12**
10	Tax@ 30%	0.00	3.11	21.03	24.76	29.92	34.68	36.54	36.93	37.26	37.54
11	**Profit after Tax (PAT)**	**-12.21**	**7.27**	**49.06**	**57.77**	**69.82**	**80.92**	**85.26**	**86.18**	**86.94**	**87.59**

12	Surplus for repayment	-12.21	45.45	82.83	86.92	92.22	96.04	94.18	93.79	93.46	93.19

Annexure-IX

REPAYMENT SCHEDULE	
Project cost	**181.23**
Bank Loan	**135.92**
Margin (@ 25 %)	**45.31**
Rate of Interest (%)	**12.00%**
Repayment period (years)	**7**

Year	**Bank Loan**	**Loan**	**Interest**	**Deferred**	**Surplus available**	**Repayment**			**Total**	**Net**	**DSCR**
	disbursed	**outstanding**		**Interest**	**for repayment**	**Interest**	**Deferred Interest**	**Principal**	**outgo**	**surplus**	
1	128.57	128.57	15.43	15.43	0.00	0.00	0.00	0.00	0.00	0.00	
2	7.35	135.92	16.31	0.00	30.56	16.31	0.00	2.03	18.34	12.22	1.67
3		133.89	16.07	0.00	60.64	16.07	15.43	4.89	36.38	24.26	1.67
4		129.01	15.48	0.00	63.56	15.48	0.00	22.65	38.13	25.42	1.67
5		106.35	12.76	0.00	67.82	12.76	0.00	27.93	40.69	27.13	1.67
6		78.42	9.41	0.00	95.70	9.41	0.00	48.01	57.42	38.28	1.67
7		29.35	3.52	0.00	94.18	3.52	0.00	29.35	32.87	61.31	2.87
											1.87